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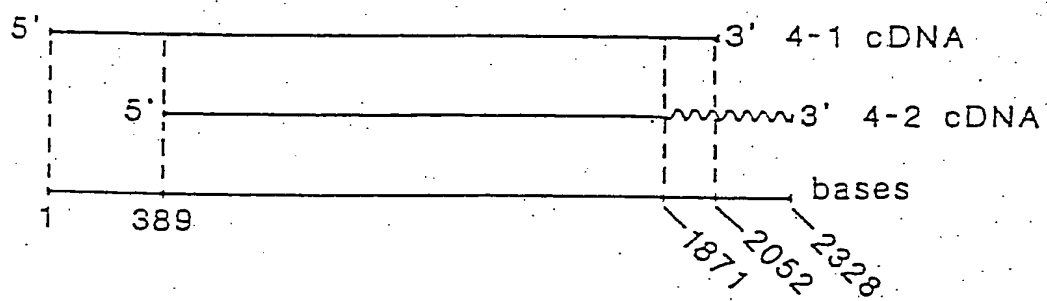


FIG. 1

66C ACC 66G 6CG CC6 CC6 CC6 CT6 CT6 30 CTA CT6 CC6 CT6 CT6 CT6 CTC 50 CTA 66G ACC 66C
 Gly Thr Gly Ala Pro Pro Pro Leu Leu Leu Leu Pro Leu Leu Leu Leu Leu Gly Thr Gly
 CTC TT6 CCT 6CT AGC AGC CAC ATA 6AG ACC 66G 6CC CAT 6CG 6AG 6AG 110 CCG CTC CT6 AAG
 Leu Leu Pro Ala Ser Ser His Ile Glu Thr Arg Ala His Ala Glu Glu Arg Leu Leu Lys
 ASA CTC TTC TCC 6GT TAC AAC AAG TGG TCT 150 CCG CCA 6TA 6GC AAT 170 ATC TCA GAT 6TG 6TC
 Arg Leu Phe Ser Gly Tyr Asn Lys Trp Ser Arg Pro Val Gly Asn Ile Ser Asp Val Val
 CTC 6TC 6CG TTT 66C TT6 TCC ATT 6CT CAG 210 CTC ATT 6AC 6TG 6AC 6AG AAG AAC CAG ATG
 Leu Val Arg Phe Gly Leu Ser Ile Ala Glu Leu Ile Asp Val Asp Glu Lys Asn Glu Met
 ATG ACA ACC AAC 6TG TGG 6TG AAG CAG 270 6AG TGG CAC 6AC TAC AAG 290 CTG 6CG TGG 6AC CCT
 Met Thr Thr Asn Val Trp Val Lys Glu Glu Trp His Asp Tyr Lys Leu Arg Trp Asp Pro
 6GT 6AC TAC 6AG AAT 6TC ACC TCC ATC 330 CCG ATC CCC TCT 6AA CTC ATC 350 TGG AGG CCT 6AC
 Gly Asp Tyr Glu Asn Val Thr Ser Ile Arg Ile Pro Ser Glu Leu Ile Trp Arg Pro Asp
 ATC 6TC CTC TAC AAC AAT 6CG 6AT 6GA 390 GAC TTT 6CA 6TC ACC CAC CTG ACC 410 AAG 6CC CAC
 Ile Val Leu Tyr Asn Asn Ala Asp Gly Asp Phe Ala Val Thr His Leu Thr Lys Ala His
 CTG TTC TAT 6AC 6GA AGG 6TG CAG TGG 450 ACA CCC CCA GCC ATC TAT AAG 470 TCC TGC AGC
 Leu Phe Tyr Asp Gly Arg Val Glu Trp Thr Pro Pro Ala Ile Tyr Lys Ser Ser Cys Ser
 ATC 6AC 6TC ACC TTC TTC CCC TTT 6AC CAG 510 CAG AAC TGT ACC ATG AAG 530 TTT GGA TCC TGG
 Ile Asp Val Thr Phe Phe Pro Phe Asp Glu Glu Asn Cys Thr Met Lys Phe Gly Ser Trp
 ACC TAC 6AC AAG GCC AAG ATT 6AC TTA 570 6TG AGC ATT CAT AGC 6GT 6TG 6AC CAA CTG 6AC
 Thr Tyr Asp Lys Ala Lys Ile Asp Leu Val Ser Ile His Ser Arg Val Asp Glu Leu Asp
 TTC TGG 6AA 6GT 66G 6AG TGG 6TC ATC 6TG 6AT 6CT 6TG 6GC ACC TAC AAC ACC AGG AAG
 Phe Trp Glu Ser Gly Glu Trp Val Ile Val Asp Ala Val Gly Thr Tyr Asn Thr Arg Lys
 TAC 6AG TGC TGT GCC 6AG ATC TAT CCT 6AC ATC ACC TAT 6CC TTC ATC ATC 710 CCA CCG CTG
 Tyr Glu Cys Cys Ala Glu Ile Tyr Pro Asp Ile Thr Tyr Ala Phe Ile Ile Arg Arg Leu
 CCG CTA TTC TAC ACC ATC AAC CTC ATC ATC 750 CCG TGC CTG CTC ATC TCC TGT 770 CTC ACC 6TG
 Pro Leu Phe Tyr Thr Ile Asn Leu Ile Ile Pro Cys Leu Leu Ile Ser Cys Leu Thr Val

FIG. 2A

790 810 830
 CTG GTC TTC TAT CTG CCT TCA GAG TGT GGC GAG AAG GTC ACA CTG TGC ATC TCG GTG CTG
 Leu Val Phe Tyr Leu Pro Ser Glu Cys Gly Glu Lys Val Thr Leu Cys Ile Ser Val Leu
 850 870 890
 CTT TCT CTC ACC GTC TTC CTG CTG CTC ATC ACC GAG ATC ATC CCG TCC ACC TCG CTG GTG
 Leu Ser Leu Thr Val Phe Leu Leu Leu Ile Thr Glu Ile Ile Pro Ser Thr Ser Leu Val
 910 930 950
 ATC CCG CTC ATC GGC GAG TAC CTC CTC TTC ACC ATG ATC TTC GTC ACC CTC TCC ATC GTG
 Ile Pro Leu Ile Gly Glu Tyr Leu Leu Phe Thr Met Ile Phe Val Thr Leu Ser Ile Val
 970 990 1010
 ATC ACG GTC TTC GTG CTC AAT GTG CAC CAC CCG TCG CCA CCG ACA CAC ACG ATG CCC GCC
 Ile Thr Val Phe Val Leu Asn Val His His Arg Ser Pro Arg Thr His Thr Met Pro Ala
 1030 1050 1070
 TGG GTG CGT AGA GTC TTC CTG GAC ATC GTG CCT CCG CTC CTC TTC ATG AAG CCG CCC TCT
 Trp Val Arg Arg Val Phe Leu Asp Ile Val Pro Arg Leu Leu Phe Met Lys Arg Pro Ser
 1090 1110 1130
 GTG GTC AAA GAC AAC TGC CCG AGA CTT ATT GAG TCC ATG CAC AAG ATG GCC AAC GCC CCC
 Val Val Lys Asp Asn Cys Arg Arg Leu Ile Glu Ser Met His Lys Met Ala Asn Ala Pro
 1150 1170 1190
 CCG TTC TGG CCA GAG CCT GTG GGC GAG CCC GGC ATC TTG AGT GAC ATC TGC AAC CAA GGT
 Arg Phe Trp Pro Glu Pro Val Gly Glu Pro Gly Ile Leu Ser Asp Ile Cys Asn Gln Gly
 1210 1230 1250
 CTG TCA CCT GCC CCA ACT TTC TGC AAC CCC ACG GAC ACA GCA GTC GAG ACC CAG CCT ACG
 Leu Ser Pro Ala Pro Thr Phe Cys Asn Pro Thr Asp Thr Ala Val Glu Thr Gln Pro Thr
 1270 1290 1310
 TGC AGG TCA CCC CCC CTT GAG GTC CCT GAC TTG AAG ACA TCA GAG GTT GAG AAG GCC AGT
 Cys Arg Ser Pro Pro Leu Glu Val Pro Asp Leu Lys Thr Ser Glu Val Glu Lys Ala Ser
 1330 1350 1370
 CCC TGT CCA TCG CCT GGC TCC TGT CCT CCA CCC AAG AGC AGC AGT GGG GCT CCA ATG CTC
 Pro Cys Pro Ser Pro Gly Ser Cys Pro Pro Pro Lys Ser Ser Ser Gly Ala Pro Met Leu
 1390 1410 1430
 ATC AAA GCC AGG TCC CTG AGT GTC CAG CAT GTG CCC AGC TCC CAA GAA GCA GCA GAA GAT
 Ile Lys Ala Arg Ser Leu Ser Val Gln His Val Pro Ser Ser Gln Glu Ala Ala Glu Asp
 1450 1470 1490
 GGC ATC CCG TGC CCG TCT CCG AGT ATC CAG TAC TGT GTT TCC CAA GAT GGA GCT GCC TCC
 Gly Ile Arg Cys Arg Ser Arg Ser Ile Gln Tyr Cys Val Ser Gln Asp Gly Ala Ala Ser
 1510 1530 1550
 CTG GCT GAC AGC AAG CCC ACC AGC TCC CCG ACC TCC CTG AAG GCC CCG CCA TCC CAG CTT
 Leu Ala Asp Ser Lys Pro Thr Ser Ser Pro Thr Ser Leu Lys Ala Arg Pro Ser Gln Leu

FIG. 2B

1570
 CCC GTG TCA GAC CAG GCC TCT CCA TGC AAA TGC ACA TGC AAG GAA CCA TCT CCT GTG TCC
 Pro Val Ser Asp Gln Ala Ser Pro Cys Lys Cys Thr Cys Lys Glu Pro Ser Pro Val Ser
 1590
 CCA GTC ACT GTG CAC AAG GCG GGA GGC ACC AAA GCA CCT CCC CAA CAC CTG CCC CTG TCA
 Pro Val Thr Val Leu Lys Ala Gly Gly Thr Lys Ala Pro Pro Gln His Leu Pro Leu Ser
 1630
 CCA GCC CTG ACA CGG GCA GTA GAA GGC GTC CAG TAC ATT GCA GAC CAC CTC AAG GCA GAA
 Pro Ala Leu Thr Arg Ala Val Glu Gly Val Gln Tyr Ile Ala Asp His Leu Lys Ala Glu
 1650
 GAC ACT GAC TTC TCG GTG AAG GAG GAC TGG AAA TAC GTG GCC ATG GTC ATT GAC CGA ATC
 Asp Thr Asp Phe Ser Val Lys Glu Asp Trp Lys Tyr Val Ala Met Val Ile Asp Arg Ile
 1670
 TTC CTC TGG ATG TTC ATC ATT GTC TGC CTT CTG GGC ACT GTG GGA CTC TTC CTG CCT CCC
 Phe Leu Trp Met Phe Ile Ile Val Cys Leu Lue Gly Thr Val Gly Leu Phe Leu Pro Pro
 1690
 TGG CTG GCT GCT TGC TGA TGGCTTCGACAGTGTTCACAGGCTCACGTCTCCTGCTGACTTTGTTTCCCAG
 Trp Leu Ala Ala Cys
 1710
 TTTCTTCTCCGACAAAGTTGGCCTCCCTTCATTTATTTCTGTTATTTTGGGCTTCGTGTTATTAATATCCTTCCCTGCC
 1730
 TCTGTGGCGCATTGTAAGTTTTAAAAATTAATAGACCAAAGCC...3'
 4-2 cDNA: 3' end
 1867
 CCC TGG CTG GCT GGT ATG ATC TAG
 Pro Trp Leu Ala Gly Met Ile
 1884
 GGACGTGGTGGTGGCCAGCTCCACATCTCTGTAGGGCCATAC
 1912
 GACTGCTCAGTCACCCACATCTTCCAAACCGGCTGACCATGAGACACCCTAGGAGAGAGATGATGCTTCTTGGGAGATG
 1937
 GAAGTTGGCCCTGGTTCTAGTCAGACTATGGGCGTGGTTGGAGAGAAATGAGGGCTGATACAGTTGCAAGCCGAGTCCC
 2016
 CATTAAAGTTTCTCCAGAGCAAGTGGCAGTACTCCTGACTTACAGACAGCACACCCATCTGTGTACAGAGAATGA
 2042
 TCCCGAAGTTGATCTCAGTTGTCTTTGAGGCCATGAAAAATTCATCCACCTTGAGGAACCAAGAGCTTCTCATGCTGTGG
 2070
 GATCAATAAGACCAGGAATCTCCCACTGTGACTCTGCTGGCCACACCCTCTCCCTCCCCAAGAAGTGGTCCCTCATCC
 2095
 CCCAATTC...3'
 2174
 2200
 2228
 2253
 2279
 2307

FIG. 2C

[illegible]

FIG. 3A

180 Asp Phe Trp Glu Ser Gly Glu Trp Val Ile Val Asp Ala Val Gly Thr Tyr Asn Thr Arg Lys Tyr Glu Cys Glu Ile Tyr Pro
 GAC TTC TGG GAA AGT GGG GAG GAG GTC ATC ATC GTC GAT GCT GCT GTG GGC 570 ACC TAC AAC ACC AGG AAG TAC GAG TGC TGT GGC GAG ATC TAT CCT
 540
 210 Asp Ile Thr Tyr Ala Phe Ile Ile Arg Arg Leu Pro Leu Phe Tyr Thr Ile Asn Leu Ile Ile Pro Cys Leu Leu Ile Ser Cys Leu Thr
 GAC ATC ACC TAT GCC TTC ATC ATC GGA GGC GGC GTC CCG CTA TTC TAC ACC ATC AAC CTC ATC ATC CCG TGC CTC CTC ACC
 630
 240 Val Leu Val Phe Tyr Ile Pro Ser Glu Cys Gly Gly Val Lys Val Thr Leu Cys Ile Ser Val Leu Leu Ser Leu Thr Thr Phe Leu Leu
 GTG CTG ATC TTC TAT CTG CCT TCA GAG GAG TGT GGC GAG GAG GTC ACA CTG CTG ATC TCG GTC CTG CTT TCT CTC ACC
 720
 270 Ile Thr Glu Ile Ile Pro Ser Thr Ser Leu Val Ile Pro Leu Ile Gly Glu Tyr Leu Leu Phe Thr Met Ile Phe Val Thr Leu Ser Ile
 ATC ACC GAG ATC ATC CCG TCC ACC TCG CTG GTC ATC CCG CTC ATC GGC GAG TAC CTC CTC ACC ATG ATC TTC 870
 300 Val Ile Thr Val Phe Val Leu Asn Val His His Arg Ser Pro Arg Thr His Thr Met Pro Ala Trp Val Arg Arg Val Phe Leu Asp Ile
 GTC ATC ACG GTC TTC GTG CAC CAC CAC CGC TCG CCA CGC ACA CAC CAC ACG ATG CCC GGC GGC TGG GTG CGT AGA 960
 330 Val Pro Arg Leu Leu Phe Met Lys Arg Pro Ser Val Val Lys Asp Asn Cys Arg Arg Leu Ile Glu Ser Met His Lys Met Ala Asn Ala
 GTG CCT CGC CTC CTC ATG AAG CGC CCC TCT GTG GTC AAA GAC AAC TGC CGG AGA CTT ATT GAG TCC ATG CAC CCA CCA 1050
 360 Pro Arg Phe Trp Pro Phe Glu Gly Glu Pro Gly Ile Leu Ser Asp Ile Cys Asn Gln Gly Leu Ser Pro Ala Thr Phe Cys Asn
 CCC CGC TTC TGG CCA GAG CCT GTG GGC GAG CCC GGC ATC TTG AGT GAC ATC TGC AAC CAA GGT CTG TCA CCT GCC 1140
 390 Pro Thr Asp Thr Ala Val Glu Thr Gln Pro Thr Cys Arg Ser Pro Pro Leu Glu Val Pro Asp Leu Lys Thr Ser Glu Val Glu Lys Ala
 CCC ACG GAC ACA GCA GTC GAG ACC CAG CCT ACG TGC AGG TCA CCC CCC CTT GAG GTC CCT GAC TTG AAG ACA TCA TCA GAG GTT GAG AAG GCC
 1170
 410

FIG. 3B

[illegible]

FIG. 3C

ALPHA4 G T G A P P P L L L L P L L L L L G T G L L P A S S H
 ALPHA3 M G V V L L P P P L S M L M L V L M L L P A A S A
 ALPHA1 M E L S T V L L L L G L S S A G L V L G
 signal peptide

ALPHA4 I E T R A H A E E R L L K R L F S G Y N K W S R P V G
 ALPHA3 - - - - S E A E H R L P Q Y L F E D Y N E I I R P V A
 ALPHA1 - - - - S E H E T R L V A K L F E D Y S S V V R P V E

ALPHA4 N I S D V V L V R F G L S I A Q L I D V D E K N Q M M
 ALPHA3 N V S H P V I I Q F E V S M S Q L V K V D E V N Q I M
 ALPHA1 D H R E I V Q V T V G L Q L I Q L I N V D E V N Q I V

ALPHA4 T T N V W V K Q E W H D Y K L R W D P G D Y E N V T S
 ALPHA3 E T N L W L K Q I W N D Y K L K W K P S D Y Q G V E F
 ALPHA1 T T N V R L K Q Q W V D Y N L K W N P D D Y G G V K K

ALPHA4 I R I P S E L I W R P D I V L Y N N A D G D F A V T H
 ALPHA3 M R V P A E K I W K P D I V L Y N N A D G D F Q V D D
 ALPHA1 I H I P S E K I W R P D V V L Y N N A D G D F A I V K

ALPHA4 L T K A H L F Y D G R V Q W T P P A I Y K S S C S I D
 ALPHA3 K T K A L L K Y T G E V T W I P P A I F K S S C K I D
 ALPHA1 F T K V L L D Y T G E H I T W T P P A I F K S Y C E I I

ALPHA4 V T F F P F D Q Q N C T M K F G S W T Y D K A K I D L
 ALPHA3 V T Y F P F D Y Q N C T M K F G S W S Y D K A K I D L
 ALPHA1 V T H F P F D E Q N C S M K L G T W T Y D G S V V A I

ALPHA4 V S I H S R V D Q L D F W E S G E W V I V D A V G T Y
 ALPHA3 V L I G S S M N L K D D Y W E S G E W A I I K A P G Y K
 ALPHA1 N P E S D O P D L S N F M E S G E W V I K E A R G W X

ALPHA4 N T R K Y E C C - A E I Y P D I T Y A F I I R R L P L
 ALPHA3 H E I K Y N C C - E E I Y O D I T Y S L Y I R R L P L
 ALPHA1 H W V F Y S C C P T T P Y L D I T Y H F V M Q R L P L

ALPHA4 F Y T I N L I I P C L L I S C L T V L V F Y L P S E C
 ALPHA3 F Y T I N L I I P C L L I S F L T V L V F Y L P S D C
 ALPHA1 Y F I V N V I I P C L L F S F L T S L V F Y I P T D S
 -----MSR I----->

ALPHA4 G E K V T L C I S V L L S L T V F L L L I T E I I P S
 ALPHA3 G E K V T L C I S V L L S L T V F L L V I T E T I P S
 ALPHA1 G E K M T L S I S V L L S L T V F L L V I V E L I P S
 <-----MSR II----->

ALPHA4 T S L V I P L I G E Y L L F T M I F V T L S I V I T V
 ALPHA3 T S L V I P L I G E Y L L F T M I F V T L S I V I T V
 ALPHA1 T S S A V P L I G K Y M L F T M V F V I A S I I I T V
 -----MSR III-----

FIG. 4A

ALPHA4	F	V	L	N	V	H	E	R	S	P	R	T	H	T	M	P	A	W	V	R	R	V	F	L	D	I	V			
ALPHA3	F	V	L	N	V	H	Y	R	T	P	T	T	H	T	M	P	T	W	V	K	A	V	F	L	N	L	L			
ALPHA1	I	V	I	N	T	H	E	R	S	P	S	T	H	I	M	P	E	W	V	R	K	V	F	I	D	T	I			
----->																														
ALPHA4	P	R	L	L	F	-	-	-	M	K	R	P	S	V	V	K	D	N	C	R	R	L	I	E	S	M	H			
ALPHA3	P	R	V	M	F	-	-	-	M	T	R	P	T	S	G	E	G	D	T	P	K	T	-	-	-	-	-			
ALPHA1	P	N	I	M	F	F	S	T	M	K	R	P	S	R	D	K	Q	E	K	R	I	F	-	-	-	-	-			
----->																														
ALPHA4	X	M	A	N	A	P	R	F	W	P	E	P	V	G	E	P	G	I	L	S	D	I	C	N	O	G	L			
ALPHA3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R	T	F	Y	G	A	E	L	S	N	L	N	C	F	S	R
ALPHA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	E	D	I	D	I	S	D	I	S	G	X	P	G		
----->																														
ALPHA4	S	P	A	P	T	F	C	N	P	T	D	T	A	V	E	T	Q	P	T	C	R	S	P	P	L	E	V			
ALPHA3	A	D	S	X	S	C	K	E	G	Y	P	C	O	D	G	T	C	C	Y	C	H	H	R	R	V	K	I			
ALPHA1	P	P	P	M	G	F	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
----->																														
ALPHA4	P	D	L	K	T	S	E	V	E	K	A	S	P	C	P	S	P	G	S	C	P	P	P	K	S	S	S			
ALPHA3	S	N	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	A	N	L	T	R	S	S	S			
ALPHA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
----->																														
ALPHA4	G	A	P	M	L	I	K	A	R	S	L	S	V	Q	H	V	P	S	S	Q	E	A	A	E	D	G	I			
ALPHA3	S	E	S	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
ALPHA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
----->																														
ALPHA4	R	C	R	S	R	S	I	Q	Y	C	V	S	Q	D	G	A	A	S	L	A	D	S	K	P	T	S	S			
ALPHA3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
ALPHA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
----->																														
ALPHA4	P	T	S	L	K	A	R	P	S	Q	L	P	V	S	D	Q	A	S	P	C	K	C	T	C	K	E	P			
ALPHA3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
ALPHA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
----->																														
ALPHA4	S	P	V	S	P	V	T	V	L	K	A	G	G	T	K	A	P	P	Q	H	L	P	L	S	P	A	L			
ALPHA3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	A	V	L	S	L	S	A	L	S	P	E	I		
ALPHA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	P	L	I	X	H	P	E	V			
<-----																														
ALPHA4	T	R	A	V	E	C	V	Q	Y	I	A	D	H	L	K	A	E	D	T	D	F	S	V	K	E	D	H			
ALPHA3	K	E	A	I	Q	S	V	X	Y	I	A	E	N	M	K	A	Q	N	V	A	K	E	I	O	D	D	W			
ALPHA1	K	S	A	I	E	C	V	K	Y	I	A	E	T	M	K	S	D	Q	E	S	N	N	A	A	E	E	W			
---phipathic helix----->																														
ALPHA4	K	Y	V	A	M	V	I	D	R	I	F	L	W	M	F	I	I	V	C	L	L	G	T	V	G	L	F			
ALPHA3	K	Y	V	A	M	V	I	D	R	I	F	L	W	V	F	I	L	V	C	I	L	G	T	A	G	L	F			
ALPHA1	K	Y	V	A	M	V	M	D	H	I	L	L	G	V	F	M	L	V	C	L	I	G	T	L	A	V	F			
<-----MSR IV----->																														
ALPHA4	L	P	P	W	L	A	G	M	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
ALPHA3	L	Q	P	L	M	A	-	R	D	D	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
ALPHA1	A	G	R	L	I	E	L	H	Q	Q	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
-->																														

FIG. 4B

FIG. 5A

Clone 4-1;
Antisense

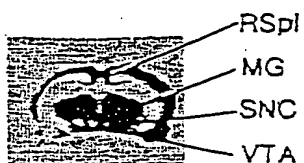
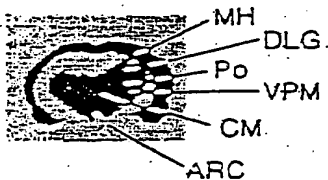
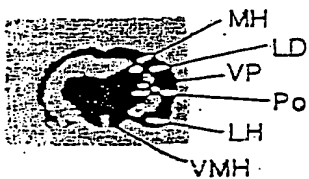
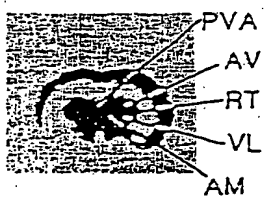
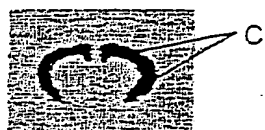


FIG. 5B

Clone 4-1;
Sense

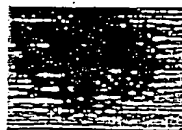
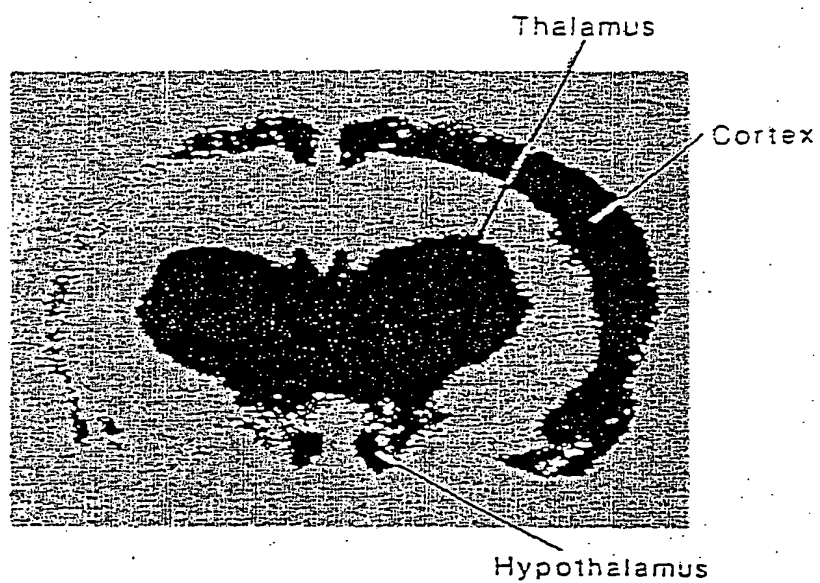
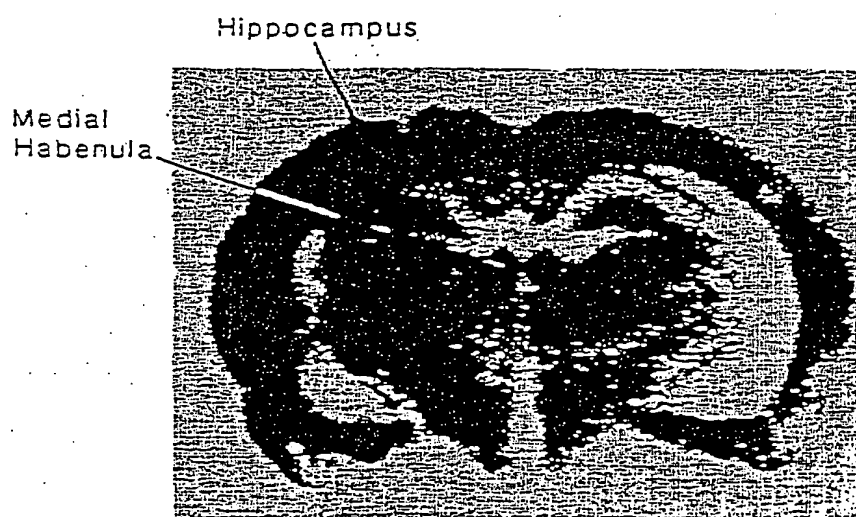


FIG. 6A



PROBE: Alpha 4

FIG. 6B



PROBE: Alpha 3

FIG. 7A

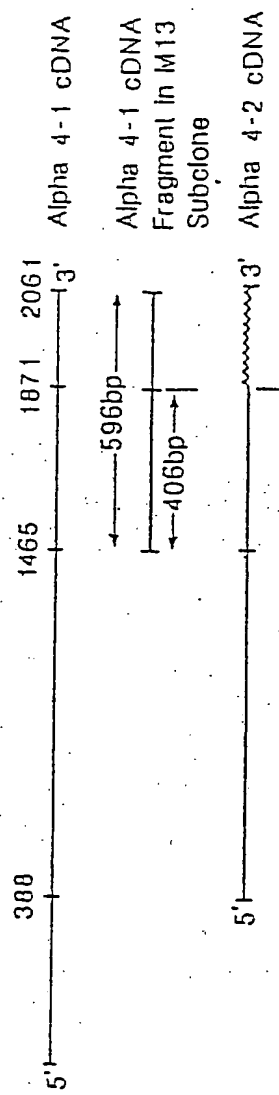


FIG. 7B

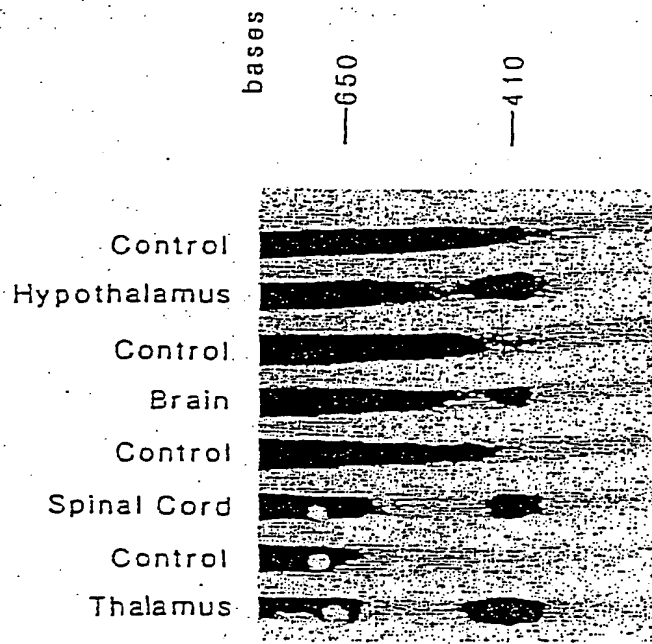
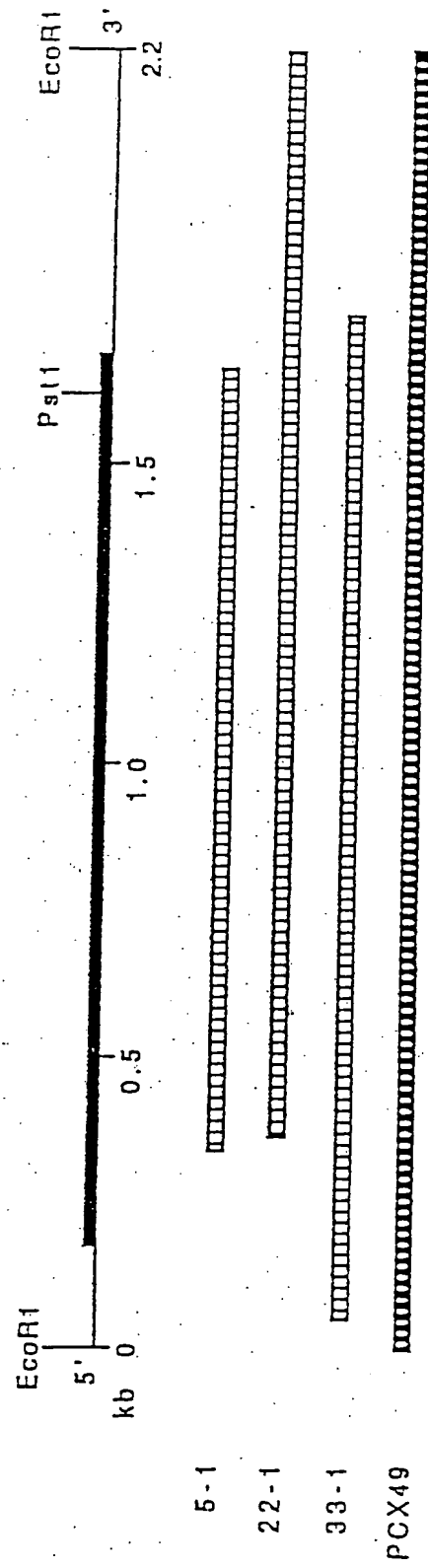


FIG. 8



-179
 5' GGGGAACACACACCGGGACCGGGAAGAGCGGGACCTCCCTCGTTGCAGGAACCTGCCCGTTTCAGTGAGCACCTTTAGACC
 -100
 TGGAGGCCGCGAGCCCAACCCCGGGAGCAGGGCGGCTTCAGCGGGCTTCAGCACCGGACAGCGGCTCGACCCCGCAGCCCTAGTATCCGAGAGGCTGCGCGCT
 -1
 ATG CTG GCT TGC ATG GCC CAC TCC AAC TCA ATG GCG CTG TTC AGC CTT CTT TGG CTG TGC TCA GGG
 Met Leu Ala Cys Met Ala Gly His Ser Asn Ser Met Ala Leu Phe Ser Phe Ser Leu Leu Trp Leu Cys Ser Gly
 1
 GTT TTG GGA ACT GAC ACA GAG GAG CCG CTA GTG GAG CAT CTC TTA GAT CCC TCC CGC IAT AAC AAG CTG ATT CGT
 Val Leu Gly Thr Asp Thr Glu Glu Arg Leu Val Glu His Leu Leu Asp Pro Ser Arg Tyr Asn Lys Leu Ile Arg
 26
 CCA GCT ACT AAC GGC TCT GAG CTG GAG CTG ACT GTA CAG CTC ATG GTA TCA TTG GCT CAG CTC ATT AGT GTG CAC GAG
 Pro Ala Thr Asn Gly Ser Glu Leu Val Thr Val Gln Leu Met Val Ser Leu Ala Gln Leu Ile Ser Val His Glu
 51
 CCG GAG CAG ATC ATG ACC ACC AAT GTC TGG CTG ACC CAG GAG TGG GAA GAT TAC CGC CTC ACA TGG AAG CCT GAG
 Arg Glu Gln Ile Met Thr Thr Asn Val Trp Leu Thr Gln Glu Trp Glu Asp Tyr Arg Leu Thr Trp Lys Pro Glu
 76
 GAC TTC GAC AAT ATG AAG AAA GTC CCG CTC CCT TCC AAA CAC ATC TGG CTC CCA GAT GTG GTT CTA TAC AAC AAT
 Asp Phe Asp Asn Met Lys Lys Val Arg Leu Pro Ser Lys His Ile Trp Leu Pro Asp Val Val Leu Tyr Asn Asn
 101
 GCT GAC GGC ATG TAC GAA GTC TCC TTC TAT TCC AAT GCT GTG GTC TCC IAT GAT GGC AGC ATC TTT TGG CTA CCA
 Ala Asp Gly Met Tyr Glu Val Ser Phe Tyr Ser Asn Ala Val Val Ser Tyr Asp Gly Ser Ile Phe Trp Leu Pro
 126
 CCT GCC ATC TAC AAG AGT GCA TGC AAG ATT GAG GTG AAG CAC TTC CCA TTT GAC CAG AAT TGC ACC ATG AAG
 Pro Ala Ile Tyr Lys Ser Ala Cys Lys Ile Glu Val Lys His Phe Pro Phe Asp Gln Gln Asn Cys Thr Met Lys
 151

FIG. 9A

540
 TTT CGC TCA TGG ACC TAC GAC CGT ACT GAG ATT GAC CTG GTG CTC AAA AGT GAT GTG GCC AGT CTG GAT GAC TTC
 Phe Arg Ser Trp Thr Thr Tyr Asp Arg Thr Glu Ile Asp Leu Val Leu Lys Ser Asp Val Ala Ser Leu Asp Phe
 176

690
 ACA CCC AGC GGG GAG TGG GAC ATC ATC GCA CTG CCA GGC CGA CGC AAC GAG AAC CCA GAC GAC TCC ACC TAT GTG
 Thr Pro Ser Gly Glu Thr Asp Ile Ile Ile Ala Leu Pro Gly Arg Arg Asn Glu Asn Pro Asp Asp Ser Thr Tyr Val
 201

690
 GAC ATC ACC TAT GAC TTC ATC ATT CGT CGC AAA CCA CTC TTC TAC ACT ATC AAC CTC ATC ATC CCC TGC GTA CTC
 Asp Ile Thr Tyr Asp Phe Ile Ile Arg Arg Lys Pro Leu Phe Tyr Thr Ile Asn Leu Ile Ile Pro Cys Val Leu
 226

780
 ATC ACC TCG CTG GCC ATC CTG GTC TTC TAC TAC GAC TGT GGT GAA AAG ATG ACA CTT TGT ATT TCT GTG
 Ile Thr Ser Leu Ala Ile Leu Val Phe Tyr Tyr Leu Pro Ser Asp Cys Gly Glu Lys Met Thr Leu Cys Ile Ser Val
 251

840
 CTG CTA GCA CTC ACG GTG TTC CTG CTC ATC TCC AAG ATT GTG CCT CCC ACC TCC CTC GAT GTA CCG CTG GTG
 Leu Leu Ala Leu Thr Thr Val Phe Leu Leu Ile Ser Lys Ile Val Pro Pro Thr Ser Leu Asp Val Pro Leu Val
 276

930
 GGC AAG TAC CTC ATG TTT ACC ATG GTG CTA GTC ACC TTC TCC ATC GTC ACC AGC GTG TGT GTG CTC AAT GTG CAC
 Gly Lys Tyr Leu Met Phe Thr Met Val Leu Leu Val Thr Phe Ser Ile Val Thr Ser Val Cys Val Leu Asn Val His
 301

990
 CAC CGC TCG CCT ACC ACG CAC ACC ATG GCC CCC TGG GTC AAG GTG GTC TTC CTG GAG AAG AAG CTC CCC ACC CTG CTC
 His Arg Ser Pro Thr Thr Thr His Thr Met Ala Pro Trp Val Lys Val Val Phe Leu Glu Lys Leu Pro Thr Leu Leu
 326

1080
 TTC CTG CAG CAG CCA CGC CAC CGC TGT GCA CGT CAG CGT CTG CGC TTG AGG AGG CGC CAG CGA GAG CGT GAG GGC
 Phe Leu Gln Gln Pro Arg Arg His Arg Cys Ala Arg Gln Arg Leu Arg Arg Arg Arg Gln Arg Glu Arg Gly
 351

FIG. 9B

1140 GAG GCG GTT TTC TTC CGT GAA GGT CCT GCG GCT GAC CCA TGT ACC TGC TTT GTC AAC CCT GCA TCA GTG CAG GGC 1200
 Glu Ala Val Phe Phe Arg Glu Gly Pro Ala Ala Asp Pro Cys Thr Cys Phe Val Asn Pro Ala Ser Val Gln Gly
 376
 1230 TTG GCT GGG GCT TTC CGA GCT GAG CCC ACT GCA GCC GGC CCG GGG CGC TCT GTG GGG CCA TGC AGC TGT GGC CTC
 Leu Ala Gly Ala Phe Arg Ala Glu Pro Thr Ala Ala Gly Pro Gly Arg Ser Val Gly Pro Cys Ser Cys Gly Leu
 401
 1290 CCG GAA GCA GTG GAT GGC GTA CGC TTC ATT GCG GAC CAC ATG CGA AGT GAG GAT GAT GAC CAG AGT GTG AGG GAG 1350
 Arg Glu Ala Val Asp Gly Val Arg Phe Ile Ala Asp His Met Arg Ser Glu Asp Asp Arg Val Arg Glu
 426
 1380 GAC TGG AAA TAC GTT GCC ATG GTG ATC GAC CGC CTG TTC CTG TGG ATC TTT GTC TTT GTC TGT GTC TTT GGG ACC
 Asp Trp Lys Tyr Val Ala Met Val Ile Asp Arg Leu Phe Leu Trp Ile Phe Val Phe Val Cys Val Phe Gly Thr
 451
 1440 GTC GGC ATG TTC CTG CAG CCT CTC TTC CAG AAC TAC ACT GCC ACT ACC TTC CTC CAC CCT GAC CAC TCA GCT CCC 1500
 Val Gly Met Phe Leu Gln Pro Leu Phe Gln Asn Tyr Thr Ala Thr Thr Phe Leu His Pro Asp His Ser Ala Pro
 476
 1596 AGC TCC AAG TGAGGTCATTCATTTCAGCTCCTCACCCCGTGACCCCTGGGTTTCAGTACTGGGTGCAAGATGGATCTCTCCCCACTCCACTGA
 Ser Ser Lys
 501
 1695 AGCCTGCTTCACACCCTCCGTTACACATAGTCCTCCAGCCTGGAGGCTGGACCCGGCTGGCTTGTGGTCGAGCCCTTCTCCTTTCCCTCTGAGCTCGTTCA
 GGCAGGAGTGCCCAATGGTGGGGCCACGGCTGGTAAGTAGAGGCCAGAGATCACAGAGGCCACCTACCCCCGATGAGGTGCTGGAGAAAGGCCCAAGAAAG
 1794
 1893 AGACAGAGTTATCTGTGACCTCCAAAGTCATCGGAGAGGAGGAGGTAGGATAAGGGGCTCAGACTCTGGCAGAGTGGGGCTAGTACTTGGCGCCCACTCA
 1992 CTTAAGTGAGCGACACTGGTCTGGGAGGACTCGAAGTGTGGGAGGCTCCTTGGGAGGCTCGGTCTCCACCCTGTACCTCAGAGGGCTCCAGACCCCGG
 2017
 GCTTCAGGTTCCCTTCTGCCAGTGC.....3'

FIG. 9C

NEURONAL AND MUSCLE NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNITS

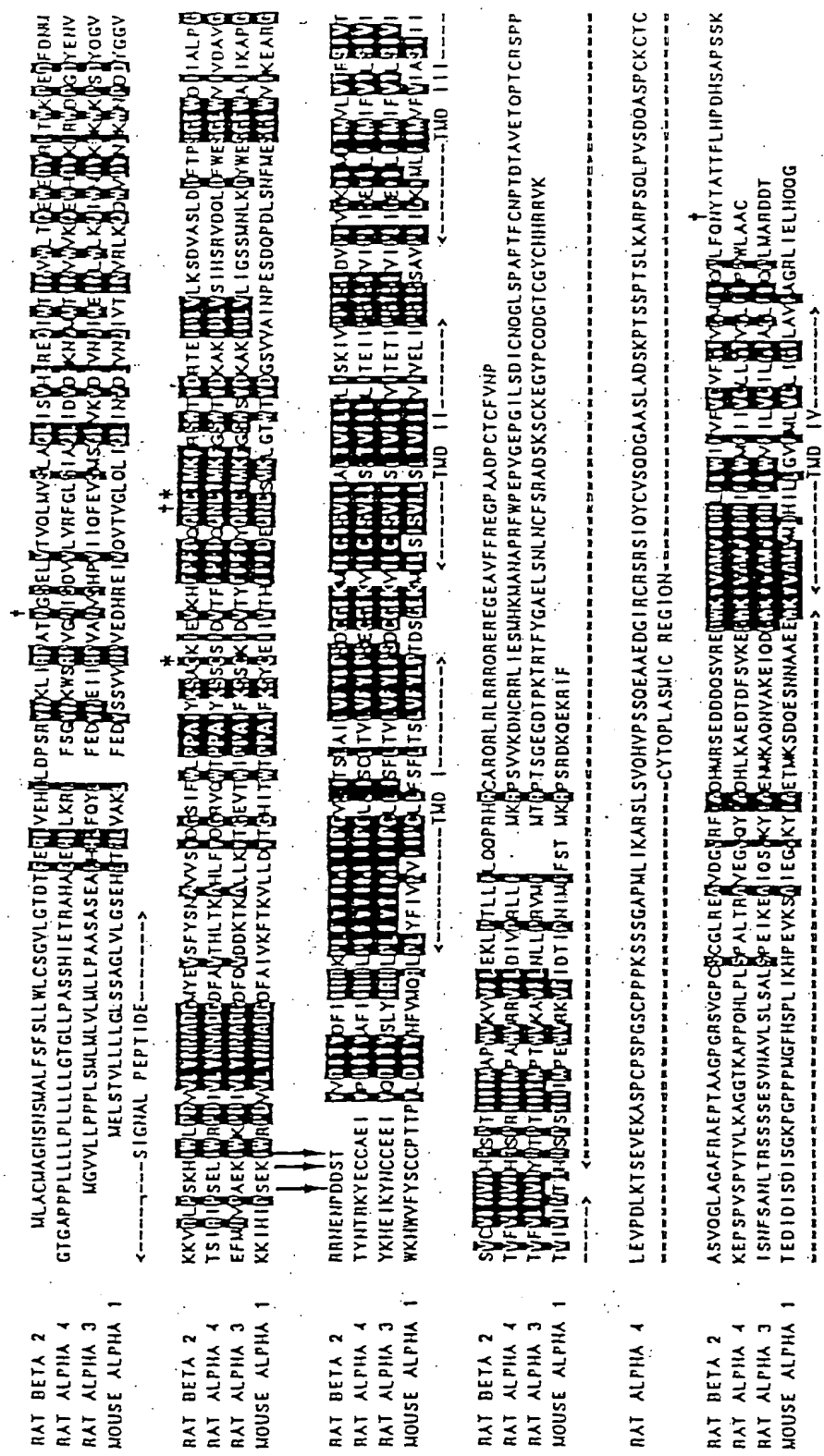


FIG. 10

FIG. 11A



—28S

—18S

FIG. 11B

1

2



28S—

18S—

FIG. 12A

ANTISENSE

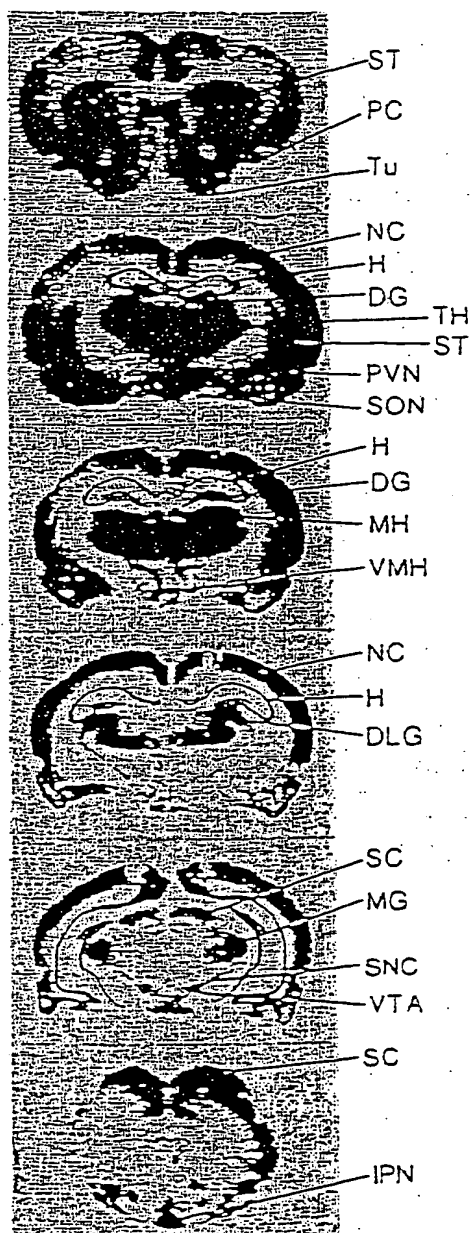


FIG. 12B

SENSE



[illegible]

FIG. 13

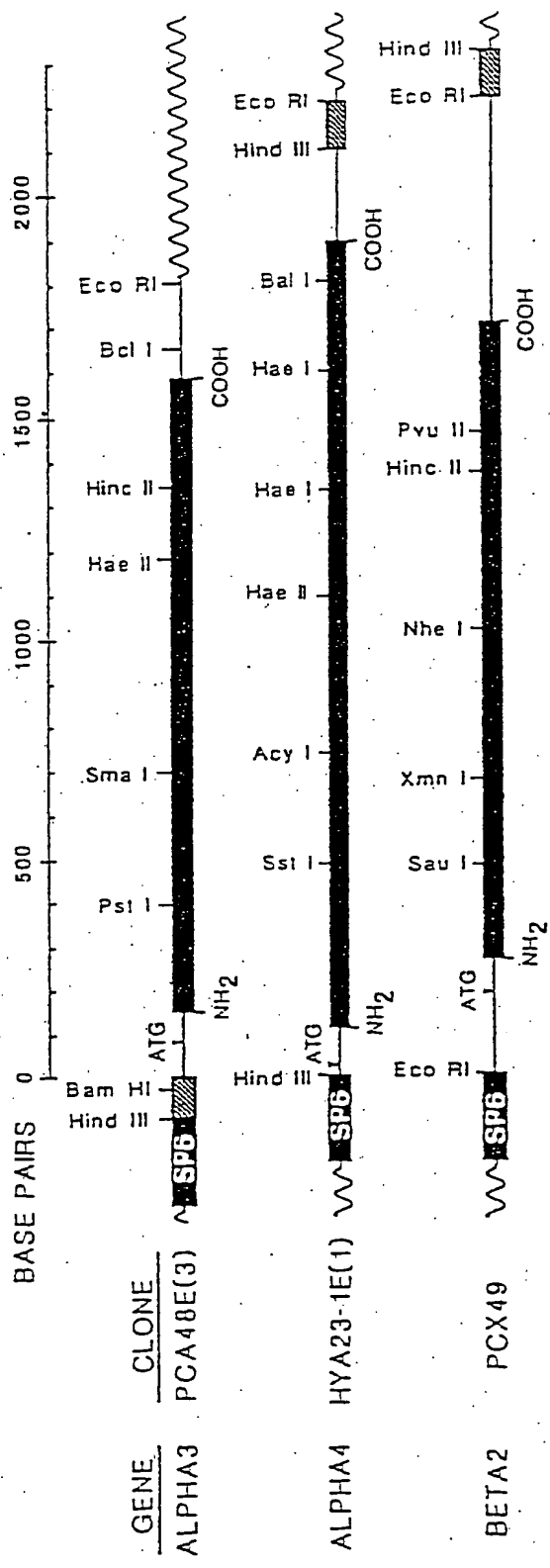


FIG. 14

FIG. 15A

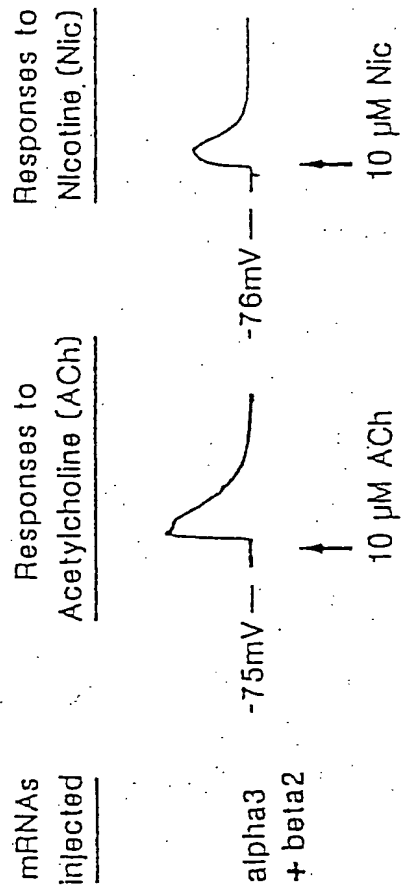


FIG. 15B

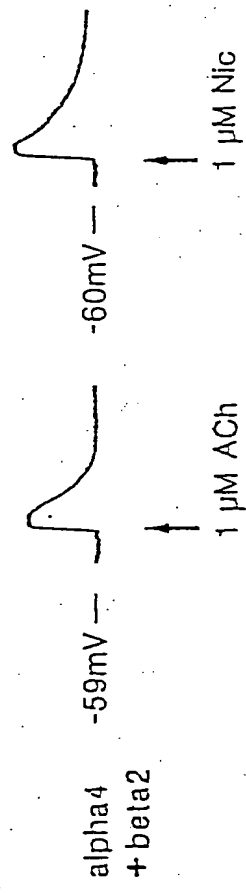


FIG. 15C

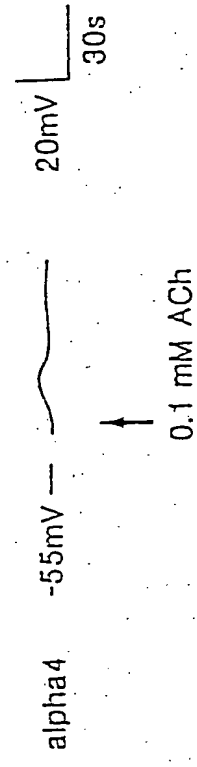


FIG. 16A

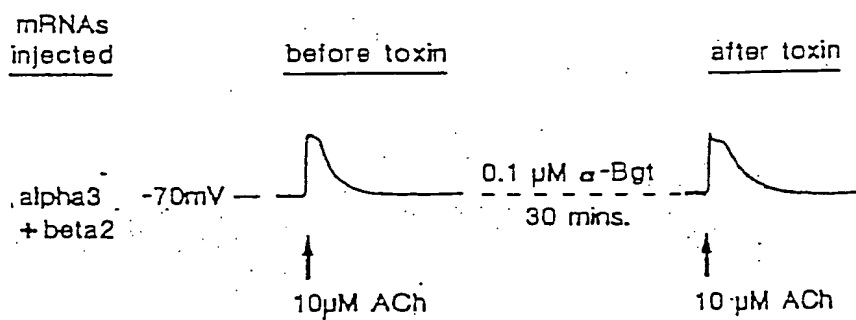


FIG. 16B

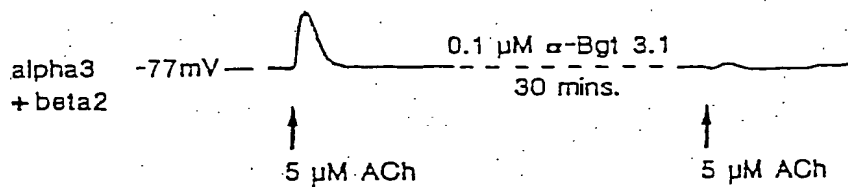


FIG. 16C

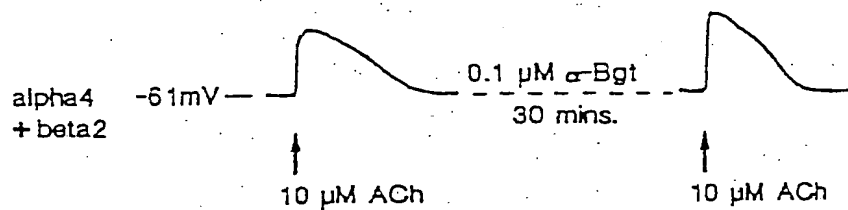
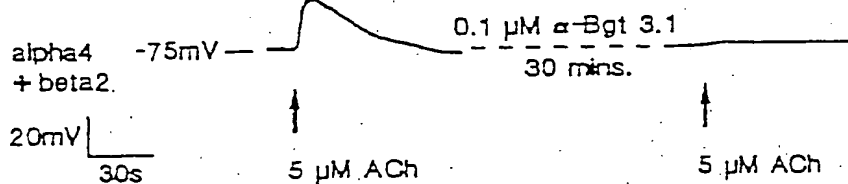


FIG. 16D



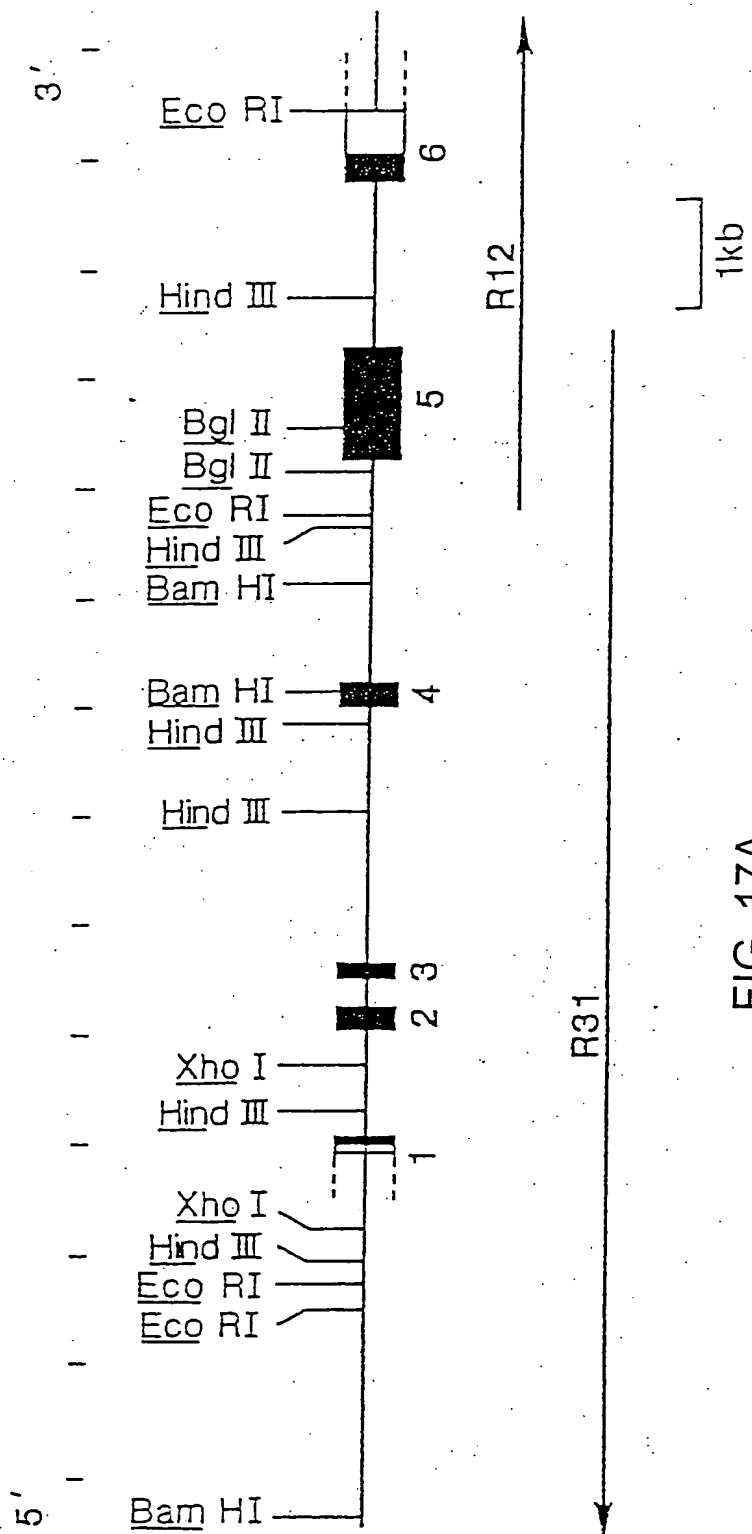


FIG. 17A

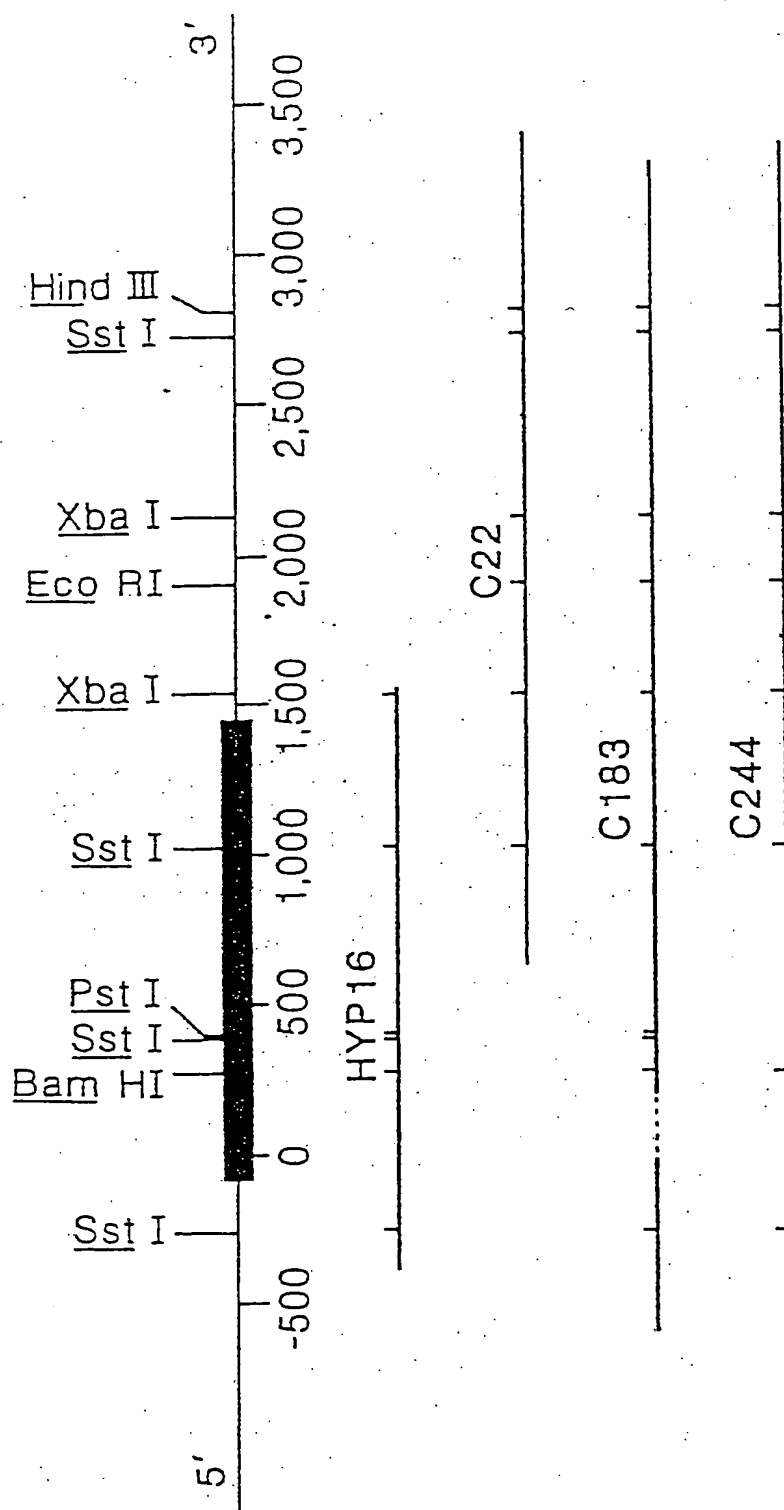


FIG. 17B

5'ACTGAGCATTTC

-350 -330 -300 -270
TGTGAACTCGGATCACCTATCTCCAGGAAGCTAGCCTGAATCCCTCATCTCCCAACAGTGGCTCTCCCAACCTTGCAGGTTCTGTGCTGGGCAACCATGAGCTGAAAGCACTGAGCTCTCG

-240 -210 -180 -150
TTCTGCACCTGTCTACTGCTTCGAGGGAGCCCTCGTCAGCCACCCCAAGGTCCTCCAGCCGGTTGGTTCTCTGCATCCCTTGAGGGGCCCTGTCTTCTTATGACAAATTGCAGAGAGACACAGT

-120 -90 -60 -20
GCCTCAAGAAGCCAGCTCTTGGTAGTCCAAAGGGAAACCAAGGACCCCTCTGAAGCC ATG ACC CTT ICC CAT TCT GCT CTC CAG TTC TGG ACA CAT CTT TAT CTC
Met Thr Leu Ser His Ser Ala Leu Gln Phe Trp Thr His Leu Tyr Leu

-30 -1 -1 1 30
TGG TGT CTC CTT CTG GTG CCA GCA G gtagt.....tateccacag TG TTG ACC CAG CAA GGC TCA CAC ACC CAT GCT GAG GAC CGC CTG TTC
Trp Cys Leu Leu Val Pro Ala V al Leu Thr Gln Gln Gly Ser His Thr His Ala Glu Asp Arg Leu Phe

60 90 120
AAA CAC CTG TTT GGA GGC TAC AAT CGC TGG GCA CGG CCA GTG CCC AAC ACT TCT GAT GTG GTG ATC GTG CGC TTT GGA TTA ICC ATT GCT
Lys His Leu Phe Gly Gly Tyr Asn Arg Trp Ala Arg Pro Val Pro Asn Thr Ser Asp Val Ile Val Arg Phe Gly Leu Ser Ile Ala

150 180
CAG CTC ATA GAT GTG gtgggc.....gctacaacag GAT GAG AAG AAT CAA ATG ATG ACC ACC AAT GTG TGG CTA AAG CAG gtaaac.....
Gln Leu Ile Asp Val Asp Glu Lys Asn Gln Met Thr Thr Thr Asn Val Trp Leu Lys Gln

210 240
.....ccctaagcag GAA TGG AAT GAC TAC AAG CTG CGC TGG GAC CCG GCT GAG TTT GGC AAT GTC ACC TCC CTG CGC GTC CCT TCA GAG ATG
Glu Trp Asn Asp Tyr Lys Leu Tyr Lys Arg Trp Asp Pro Ala Glu Phe Gly Asn Val Thr Ser Leu Arg Val Pro Ser Glu Met

FIG. 18A

270 ATC TGG ATC CCA GAC AAT GTC CTC TAC AAC AA gtaaga.....ctcttccag T 300 GCA GAT GGG GAG TTT GCG GTG ACC CAC ATG ACC AAG
 ile Trp ile Pro Asp ile Val 100 n 100 Ala Asp Gly Glu Phe Ala Val Thr His Met Thr Lys
 90
 360 GCT CAC CTC TTC TTC ACG GGC ACT Thr Val His Trp Val Pro Pro Ala ile Tyr 390 AGC TCC TGC AGC ATC GAT GTG ACC TTC CCC TTC
 Ala His Leu Phe Phe Thr Gly 120 Thr Val 130 Lys Ser Ser Cys Ser ile Asp Val Thr Phe 140 TTC CCC TTC
 450 GAC CAG CAG AAC TGC AAG ATG AAG Lys Phe Gly Ser Trp Thr Tyr Asp Lys Ala 160 ATC GAT CTG GAG CAG ATG GAG AGG ACA GTG GAC CTG
 Asp Gln Gln Asn Cys Lys Met 150 Lys 160 Lys ile Asp Leu Glu Gln Met Glu Arg Thr Val Asp Leu 170
 540 AAG GAC TAC TGG GAG AGT GGC GAG Trp Ala ile ile Asn Ala Thr Gly Thr 570 TAT AAC AGT AAG AAG TAC GAC TGC TGC GCG GAG ATC TAC
 Lys Asp Tyr Trp Glu Ser Gly 180 Trp 190 Tyr Asn Ser Lys Lys Tyr Asp Cys Cys Ala 200 Glu ile Tyr
 630 CCC GAT GTC ACC TAC TTT GTC ATC CCG CCG CTG CCG CTC TTC TAT ACC 660 AAC CTC ATC ATC CCA TGC CTG CTC ATC ICC TGC CTC
 Pro Asp Val Thr Tyr Tyr Phe Val 210 ile Arg Arg Leu Pro Pro Leu Phe Tyr Thr 220 ile Asn Leu ile ile Pro Cys Leu Leu ile Ser Cys Leu 230
 720 ACT GTG CTC GTG TTC TAC CTG CCT Thr Val Leu Val Phe Ser Glu Cys Gly Glu Lys ile Thr Leu 750 ATC TCG GTG CTG CTA TCT CTC ACT CTC TTC CTG CTG
 Thr Val Leu Val Phe Tyr Leu 240 Pro 250 Cys 260 Phe 270 Phe 280
 810 CTC ATC ACG GAG ATC ATC CCG TCC ACC TCG CTG GTC ATC CCA CTC ATC GGC GAG Thr Ser Leu Val ile Pro Leu ile Gly 840 TAC CTG CTC TTC ACC ATG ATC TTT GTC ACC CTC TCT
 Leu ile Thr Glu ile ile Pro 270 Thr 280 Glu 290 Thr 300 Met 310 Phe 320 Val 330 Thr 340 Ser 350

FIG. 18B

ATC GTT ATC ACA GTC TTC GTG CTC AAT GTA CAC CAC CGC TCC CCC AGC ACC ACC ATG CCC AAC TGG GTA AGG GTA GCC CTG CTA GGC
 Ile Val Ile Thr Val Phe Val Leu Asn Val His His Arg Ser Pro Ser Thr His Asn Met Pro Asn Trp Val Arg Val Ala 960
 900 300

CGG GTG CCC AGG TGG CTG ATG ATG AAC CGG CCC CTG CCA CCT ATG GAG CTC CAT GGC TCC CCG GAT CTG AAG CTC AGC CCC TCA TAC CAT
 Arg Val Pro Arg Trp Leu Met Met Asn Arg Pro Leu Pro Pro Met Glu Leu His Gly Ser Pro Asp Leu Lys Leu Ser Pro Cys Tyr His 1050
 990 330

TGG CTA GAG ACT AAC ATG GAT GCT GGA GAA AGG GAG GAG ACA GAG GAA GAG GAG GAA GAA GAT GAA AAC ATA TGT GTG TGT GCA GGC
 Trp Leu Glu Thr Asn Met Asp Ala Gly Glu Arg Glu Glu Thr Glu Glu Glu Glu Asp Glu Asn Ile Cys Val Cys Ala Gly 1100
 1080 360

CTT CCA GAC TCT TCG ATG GGT GTC CTC TAT GGC CAT GGC GGC CTG CAT CTG AGA GCC ATG GAG CCT GAG ACC AAG ACT CCA TCC CAG GCT
 Leu Pro Asp Ser Ser Met Gly Val Leu Tyr Gly His Gly Gly Leu His Leu Arg Ala Met Glu Pro Glu Thr Lys Thr Pro Ser Gln Ala 1230
 1170 390

AGC GAG ATT CTG TCA CCT CAA ATA CAG AAA GCA CTA GAA GGT GTA CAC TAC ATT GCT GAC CGT CTG AGG TCT GAG GAT GCT GAC TCT
 Ser Glu Ile Leu Leu Ser Pro Gln Ile Gln Lys Ala Leu Glu Gly Tyr His Tyr Ile Ala Asp Arg Leu Arg Ser Glu Asp Ala Asp Ser 1320
 1260 420

TCG gtgaat.....ctaacttcag GTG AAG GAA GAC IGG AAG TAT GTG GCC ATG GTG GTA GAC CGG ATA TTC CTC TGG CTG TTC ATT ATC
 Ser Val Lys Glu Asp Trp Lys Tyr Val Ala Met Val Val Asp Arg Ile Phe Leu Trp Leu Phe Ile Ile 1380
 1350 450

GTC TCG TTC CTG GGG ACC ATC GGA CTC TTC CTT CCT CCA TTC CTG GCT GGA ATG ATC TAA CTTCATGTCCTTCATGTTGGCTCCAGGTGGCCTTCGTA
 Val Cys Phe Leu Thr Ile Gly Leu Phe Leu Pro Pro Phe Leu Ala Gly Met Ile 1440
 1410 470

1500
 ACTATCTTCTAGTCTCTGTGANTGGAGCCATCTCTAGATACTCTTTTGAC.....3'

FIG. 18C

[illegible][illegible][illegible][illegible][illegible]

FIG. 19

MEMBRANE SPANNING IV

ALPHA1 QESNHAAEEMSPDAVILVYVLLQLITLAVNLAGRLIELHGOO
ALPHA2 DADSSVXEDMSLVNVAIFVFLITGLLPPPLAGMI
ALPHA3 NYAKETODDMSPAVILRFVYVLLQLITLAVNLAGRLIELHGOO
ALPHA4 DIDSFVXEDMSLVNVAIFVFLITGLLPPPLAGMI

SPLIKHNEVKSIEGKTRMETUKSD
SEILLSIOXALEGHTADRLRSE
SLSALSNEIKEAIOSKCIENHUKAO
OHLPLSPALTRAVEGSCIOHLKAE
AMPHIPATHIC HELIX

FIG. 19

QESHNAAEEMSPAMALHILVYVJLHILAVLHGRILHLOOG
DADSSVYEDMILUNVIAFWLITLGLLPPFLAGMI
HYAKETODDMSPAULIRFVWGLILGLLOOPLUARDOT
DIDFSVYEDMSPAULIRFVWGLILGLLOOPLXLAGMI

MEMBRANE SPANNING IV

FIG. 20A

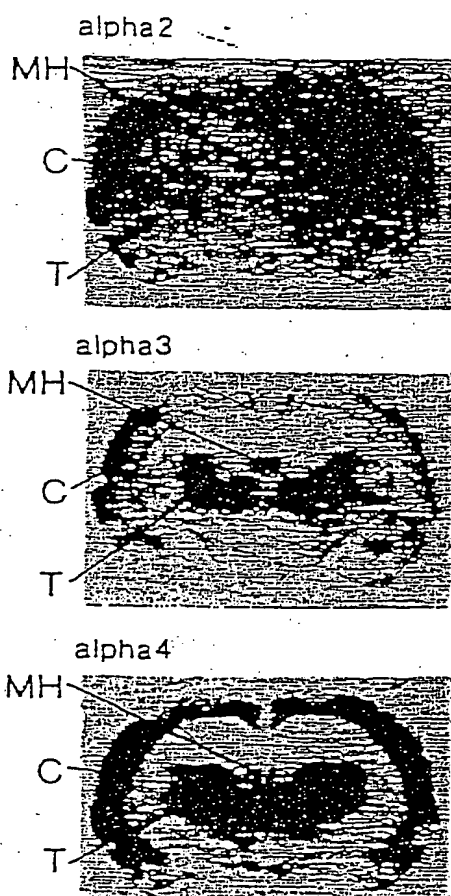
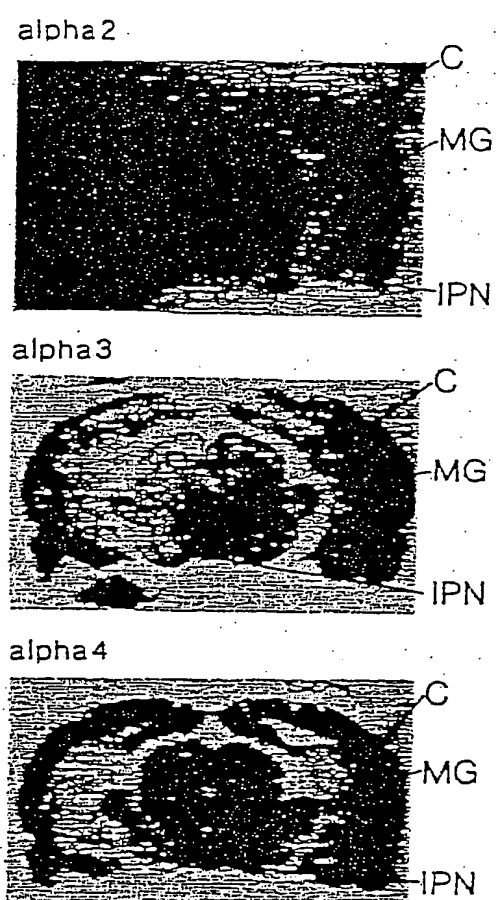


FIG. 20B



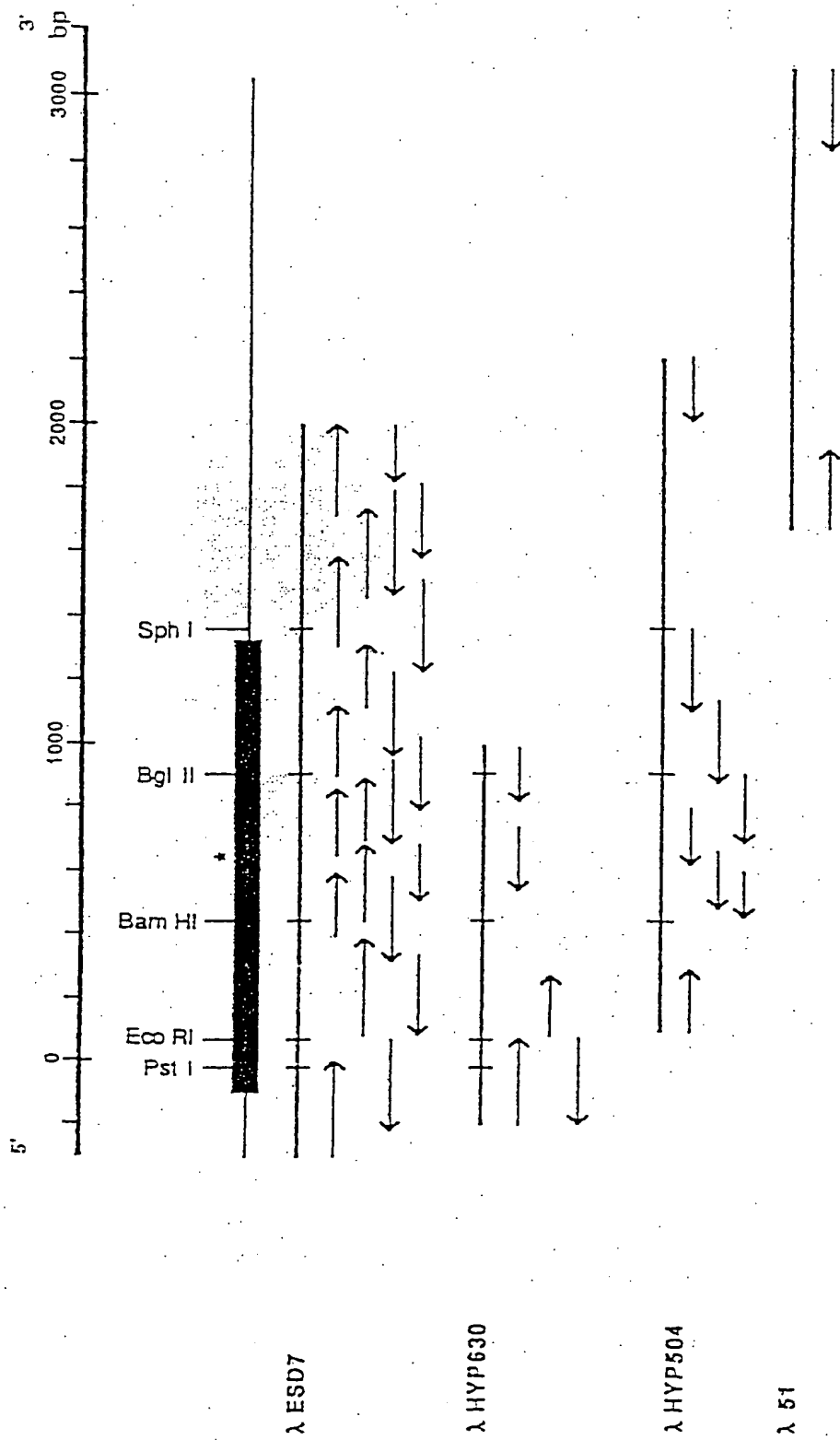


FIG. 21A

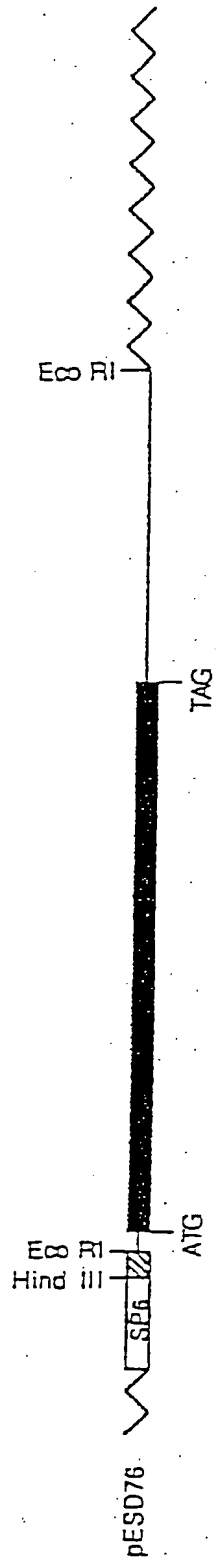


FIG. 21B

5'GACAGACATT

-240 -220 -200 -180 -160 -140

TGTGGTCATCAGCTGATTATTTTCATCAGGAGCTGGTCCCTGGTTCATCAGGCTTTGAACCACTCACATTTTGTGTTTAAACCCCTGATCCTTCCAGTGGAAACACT

-120 -100 -80 -60 -40

CTGCGCTTCAAGGAAATGTCTCTCTGAAGCAGAGCTC ATG ACA GGC TTC CTA AGG GTC TTC TTG GTT CTC AGT GCC ACT CTC TCA GGT TCC TGG GTC

-20 -10

Thr Leu Thr Ala Thr Ala Gly Leu Ser Ser Asp Ile Ile Lys Val Tyr Phe Gly Leu Lys Ile Ser Gln Leu Val Asp Val Asp Gln Lys Asn Gln Leu

ACT CTT ACG GCC ACT GCA GGA CTC AGC TCA GTG GCT GAA CAC GAA GAA GAC GCA CTC CTC AGA CAT TTG TTC CAA GGT TAC CAG AAA TGG GTC

-20 -10

Arg Pro Val Leu Asn Ser Ser Asp Ile Ile Lys Val Tyr Phe Gly Leu Lys Ile Ser Gln Leu Val Asp Val Asp Gln Lys Asn Gln Leu

CGC CCT GTG TTG AAT TCC AGT GAC ATC ATA AAA GTG TAT TTT GGA TTA AAA ATA TCC CAG CTT GTG GAT GTG GAT GAA AAG AAT CAG CTG

80 100 120 140

Met Thr Thr Asn Val Tip Tip Thr Asp Gln Lys Leu Arg Tip Asn Pro Gln Glu Tyr Gly Gly Ile Asn Ser Ile Lys

ATG ACG ACA AAT GTG TGG CTG TGG CTG AAG CAG GAA TGG ACA GAC CAA AAA TTA CGC TGG AAT CGC GAA GAA TAT GGT GGA ATT AAT TCG ATA AAG

160 180 200 220 240

Val Pro Ser Glu Ser Leu Tip Leu Pro Asp Ile Val Leu Phe Glu Asn Ala Asp Gly Arg Phe Glu Gly Ser Leu Met Thr Lys Ala Ile

GTT CCA TCA GAA TCG CTC TGG CTG CCG GAC ATA GTT CTC TTT GAA AAT GCT GAC GCA CGT TTT GAG GGC TCC CTC ATG ACC AAG GCC ATT

260 280 300 320 340

Val Lys Ser Ser Gly Thr Val Ser Tip Thr Pro Pro Ala Ser Tyr Lys Ser Ser Cys Thr Met Asp Val Thr Phe Phe Pro Phe Asp Arg

GTG AAG TCC AGT GCA ACC GTC AGC TGG ACT CCT CCC GCC AGC TAC AAG AGT TCC TGC ACC ATG GAT GTC ACA TTT TTC CCG TTC GAC AGG

360 380 400 420

FIG. 22A

150 Gln Asn Cys Ser Met Lys Phe Gly Ser Trp Thr Tyr Asp Gly Thr Met Val Asp Leu Ile Asn Glu Asn Val Asp Arg Lys Asp 170
 CAG AAC TGC TCG ATG AAG TTT GGA TCC TGG ACT TAC GAC GGT ACC ATG ATG GTT GAC CTC ATT CTA ATC AAT GAA AAC GAT GAC CGG AAA GAC 500
 440 460

180 Phe Phe Asp Asn Gly Glu Trp Glu Ile Leu Asn Ala Lys Gly Met Lys Gly Asn Arg Arg Glu Gly Phe Tyr Ser Tyr Pro Phe Val Thr 200
 TTT TTT GAT AAC GGA GAG TGG GAG ATA CTC AAC GCA AAG GGG ATG AAG GGC AAC AGA AGA GAA GGC TTT TAC TCC TAT CCG TTT GTT ACC 580
 520 540 560

210 Tyr Ser Phe Val Leu Arg Arg Leu Pro Leu Phe Tyr Thr Leu Phe Leu Ile Ile Pro Cys Leu Gly Leu Ser Phe Leu Thr Val Leu Val 230
 TAC TCT TTT GTC CTG AGA CGC CTG CCC TTG TTT TAC ACC CTC TTT TTG ATA ATC CCC TGC CTG GGG TTG TCT TTT CTC ACG GTC CTG GTG 680
 620 640 *

240 Phe Tyr Leu Pro Ser Asp Glu Gly Glu Lys Leu Ser Leu Ser Thr Ser Val Leu Val Ser Leu Thr Val Phe Leu Val Ile Glu Glu 260
 TTC TAC CTA CCC TCG GAC GAA GGG GAA AAA CTC TCA TTA TCC ACC TCC GTT TTG GTC TCT TTG ACG GTG TTT CTT TTA GTG ATT GAA GAA 760
 700 720 740

270 Ile Ile Pro Ser Ser Lys Val Ile Pro Leu Ile Gly Glu Tyr Leu Leu Phe Ile Met Ile Phe Val Thr Leu Ser Ile Ile Val Thr 290
 ATA ATC CCG TCC TCT TCG AAG GTC ATC CCC CTC ATT GGC GAG TAC CTC CTC TTC ATT ATG ATT TTT GTC ACG CTG TCT ATT ATC GTC ACG 860
 800 820

300 Val Phe Val Ile Asn Val His His Arg Ser Ser Ser Thr Tyr His Pro Met Ala Pro Trp Val Lys Arg Leu Phe Leu Gln Arg Leu Pro 320
 GTT TTT CTA ATT AAT GTC CAC CAC CAC AGA TCT TCC TCA ACG TAC CAT CCC ATG GCC CCC TGG GTG AAG AGG CTG TTT CTA CAA AGA CTC CCG 940
 880 900 920

330 Arg Trp Leu Cys Met Lys Asp Pro Met Asp Arg Phe Ser Phe Pro Asp Gly Lys Glu Ser Asp Thr Ala Val Arg Gly Lys Val Ser Gly 350
 ACA TGG CTT TGC ATG AAG GAC CCC ATG GAC CGC TTC TCT TTC CCG GAT GCA AAG GAG AGT CAT ACA GCC GTG AGG GGG AAA GTC TCA GGC 1040
 980 1000 1020

FIG. 22B

Lys Arg Lys Gln Thr Pro Ala Ser Asp Gly Glu Arg Val Leu Val Ala Phe Leu Glu Lys Ala Ser Glu Ser Ile Arg Tyr Ile Ser Arg 380
 AAA AGG AAA CAG ACT CCC GCC AGC GAT GGA GAA AGA GAT CTC GTC GCT TTC CTC GAG AAG GCC TCC GAG TCC ATC AGA TAC ATT TCG AGG 1140
 1060 1100 1120
 His Val Lys Lys Glu His Phe Ile Ser Gln Val Val Gln Asp Trp Lys Phe Val Ala Gln Val Leu Asp Arg Ile Phe Leu Trp Leu Phe 410
 CAT CTG AAA AAG GAA CAC TTC ATC AGC CAG GTA GTG CAA GAC TGG AAA TTT GTG GCT CAA GTT CTG GAC CGC ATC TTC CTG TGG CTC TTT 1220
 1160 1180 1200
 Leu Ile Ala Ser Val Leu Gly Ser Ile Leu Ile Phe Ile Pro Ala Leu Lys Met Trp Ile His Arg Phe His 430
 CTG ATA GCT TCT GTG TTG GGT TCC ATT CTG ATT TTT ATT CCA GCC TTG AAG ATG TGG ATA CAT CGT TTC CAC TAG GAGCCACTCTCTGCACCCA 1320
 1240 1260 1280 1300
 TTTAGAGACATACATAGAGACAAATCCACCTTAGGACTGACAGCGGCTGGCATGCTGACAGGAGCAGAGCCATGCAATGCTAGTGGTGGCCCTTGTCTGTGGGAGCTTCTGTG 1440
 1340 1360 1380 1400 1420
 ATTGCAGGGCACTGAGAGAAATGTGGGTTTGAGTTAGTGAGATGGTGGCTGCCATTAGAGAGGTGTAGTTGGGCAATTGGAGAGGCTCTCCATGTTATATTGTGGGAGTTCTCTGAAC 1560
 1460 1480 1500 1520 1540 1560
 TACTCCCTCTGCTCATCCCTGAACGCACCTGGGGCTATGTGGTATTTCTCTAGCAGTGTGGTGAAGCCATTTTGACAATAGTTTTCAGGAAATTACGGCAGGTACAACTCTCCACACACAGG 1680
 1580 1600 1620 1640 1660 1680
 TCAAATTTGCCACTTGTCAACGAGTGTCCCAAAATAGGGTCATTGAAGATGACCTTGAATGGCTATGACAGATTCTCTAAGCCAGGTGTACTGGGAAGTTTGGCTCACTGACCTGGGAAC 1800
 1700 1720 1740 1760 1780 1800
 TTTTCGAATGCCAGGTAGGAACCTGGGGTCATTTCTAGCTTGTCTAAGGTTCCCATCAAAATAAAGTTACCCCGAGAAACAGCCATTCCCTAGTAGAAGCTGTTATTTTTCACACACATCTC 1900
 1820 1840 1860 1880 1900
 TTTTTTTTCC.....J'
 1930

FIG. 22C

NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNITS

BETA 3	MTGFLRVFLVLSATLSGWSVTLTATAGLSSVAFEDALLRHLFOGOKWYVVLSSDIIKVVYGLKISQVYVQKNAQTNVNLKDEMTDOKHRIHQEEYGGI
BETA 2	MLACHAGHSHNALFSFLLWLCGSLGDTIGERLVEHLDPRIKALIDRATIGSELVTOLVSLACLISLHIREQNTINMLTDEEYRLTKKEDFDNH
ALPHA 2	MTLSHALOFNTHLYLWCLLVPAVLTOOGSHTHADRLFKHLFGGIRWAVVPAVTSOWVIFGLSIAIDVQINDQTNVNLKDEMTDOKHRIHQEEYGGI
ALPHA 3	MGVLLPPPLSLMLVLMPLAASASEAHLRFQYLFEDNEIIRGVANSHPIIOFEVSUSKVKQVNDDEIDVQLKQINNYKIKKCKSDYOGV
ALPHA 4	MEIGGPGAPPLLLPLLLLTGTGLPASSSHIETRAHAEHLKRLFGGAKWSSHLVGLIDVVLVRFGLSIATIDVQIKNDQTNVNLKDEMTDOKHRIHQEEYGGI
	-----SIGNAL PEPTIDE----->
BETA 3	HSIKVPSSESLDIDVTENADRRFEGSLTKAIVKSSQTSVSTPPASINSSSTUDVTFFPTDRHLSWMTGRTIDGTHVQVILINENYDRKDFDNTIEWEIIIAKQ
BETA 2	KKVLPSKHIALPDVLYIADGAYEVSFSYIAVSYDGSIFALPAIYKSAKIEVAFEPDONGTAKTPRQNTIDATEIDVLSKSDVASLDOFTPSGLADQIALP
ALPHA 2	TSLRVSEEMIDIDVTYHADDEFVTHUTKAILFFITGVKAVPPAIYKSSCSIDVTFFPTDRHLSWMTGRTIDGTHVQVILINENYDRKDFDNTIEWEIIIAKQ
ALPHA 3	EFNRVDAEKIKKQDIDVYHADGOFQVODKTKALLKTYTGEVTPPPAIYKSSCSIDVTFFPTDRHLSWMTGRTIDGTHVQVILINENYDRKDFDNTIEWEIIIAKQ
ALPHA 4	TSIRIDSELIDRPDIDVYHADGOFVTHUTKAILFFITGVKAVPPAIYKSSCSIDVTFFPTDRHLSWMTGRTIDGTHVQVILINENYDRKDFDNTIEWEIIIAKQ
	-----TMD I----->
BETA 3	MKGRRR EGFYS VPFFVTSFVLRLPLETILFLIPGGLSFTTVNVLPSDEGKLSSTSVLSVLLVLTVEEIISSSKVVIITTEILQIFVLGIV
BETA 2	RRHEHPDDSTVDIIVDFIIRKXPLEVTINKLIPGGLSFTTVNVLPSDEGKLSSTSVLSVLLVLTVEEIISSSKVVIITTEILQIFVLGIV
ALPHA 2	TVNSKKYDCCAEI VPDVLYYFVIRLPLEVTINKLIPGGLSFTTVNVLPSDEGKLSSTSVLSVLLVLTVEEIISSSKVVIITTEILQIFVLGIV
ALPHA 3	YKHEIKYNGCEEI VODIYSLYIRLPLEVTINKLIPGGLSFTTVNVLPSDEGKLSSTSVLSVLLVLTVEEIISSSKVVIITTEILQIFVLGIV
ALPHA 4	TYHTRKYEGCAEI VPDITAFIRLPLEVTINKLIPGGLSFTTVNVLPSDEGKLSSTSVLSVLLVLTVEEIISSSKVVIITTEILQIFVLGIV
	-----TMD II----->
BETA 3	TVFVIRVHHSSTVYVPIAPVKKRLFQRLQRLCUKDPHIDFESFPDGK
BETA 2	SVCLVVIHHSPTTDTAPVKKVVFEXLQTLFLQOPRHQCARQRLRRROREREAGEAVFFREGPAADPCTCFVNP
ALPHA 2	TVFVIRVHHSPTTDTAPVKKVVFEXLQTLFLQOPRHQCARQRLRRROREREAGEAVFFREGPAADPCTCFVNP
ALPHA 3	TVFVIRVHHSPTTDTAPVKKVVFEXLQTLFLQOPRHQCARQRLRRROREREAGEAVFFREGPAADPCTCFVNP
ALPHA 4	TVFVIRVHHSPTTDTAPVKKVVFEXLQTLFLQOPRHQCARQRLRRROREREAGEAVFFREGPAADPCTCFVNP
	-----CYTOPLASMIC REGION----->
ALPHA 4	PLEVPDLKTSVEKASPCSPGSGPPKSSSGAPMLIKARSLVQIHVPSSOEAEDGIRCSRSIOYCVSODGAASLADSKPTSSPTSLKARPSOLPVSDOASPCXCT
	-----TMD III----->
BETA 3	ESDTAVRGKVSQKRNKOTP ASDGERVLVAFLEKASESIRYISRHVKKENHFSOVVOQWKEFAQVIRHFEWLIASVLSILIPALKWUWHIRFH
BETA 2	ASVOGLAGAFRAEPTAAG PGRSYGPGSCGLREAVDGVRFADHURSEDODSVREDNKYVALQIDFLVITVFCVFGTGYGMDIPLFONYATTATFLHPDHSAPSSK
ALPHA 2	YGHGGLHLRAHEPETKP SOASEILLSPOIOKALEGVHYADRLRSEDODSVREDNKYVALQIDFLVITVFCVFGTGYGMDIPLFONYATTATFLHPDHSAPSSK
ALPHA 3	ISHFSAHLTHSSSSESVH AVLSLSALSPEIKENIOSVKYIAEIKAKAOHWAKEIODQNSYVALQIDFLVITVFCVFGTGYGMDIPLFONYATTATFLHPDHSAPSSK
ALPHA 4	CKEPSVPSPVILKAGGTAKAPPDHLPLSPALTRAVEGVQYADHLKAEDTDFSVKEENKYVALQIDFLVITVFCVFGTGYGMDIPLFONYATTATFLHPDHSAPSSK
	-----TMD IV----->

FIG. 23

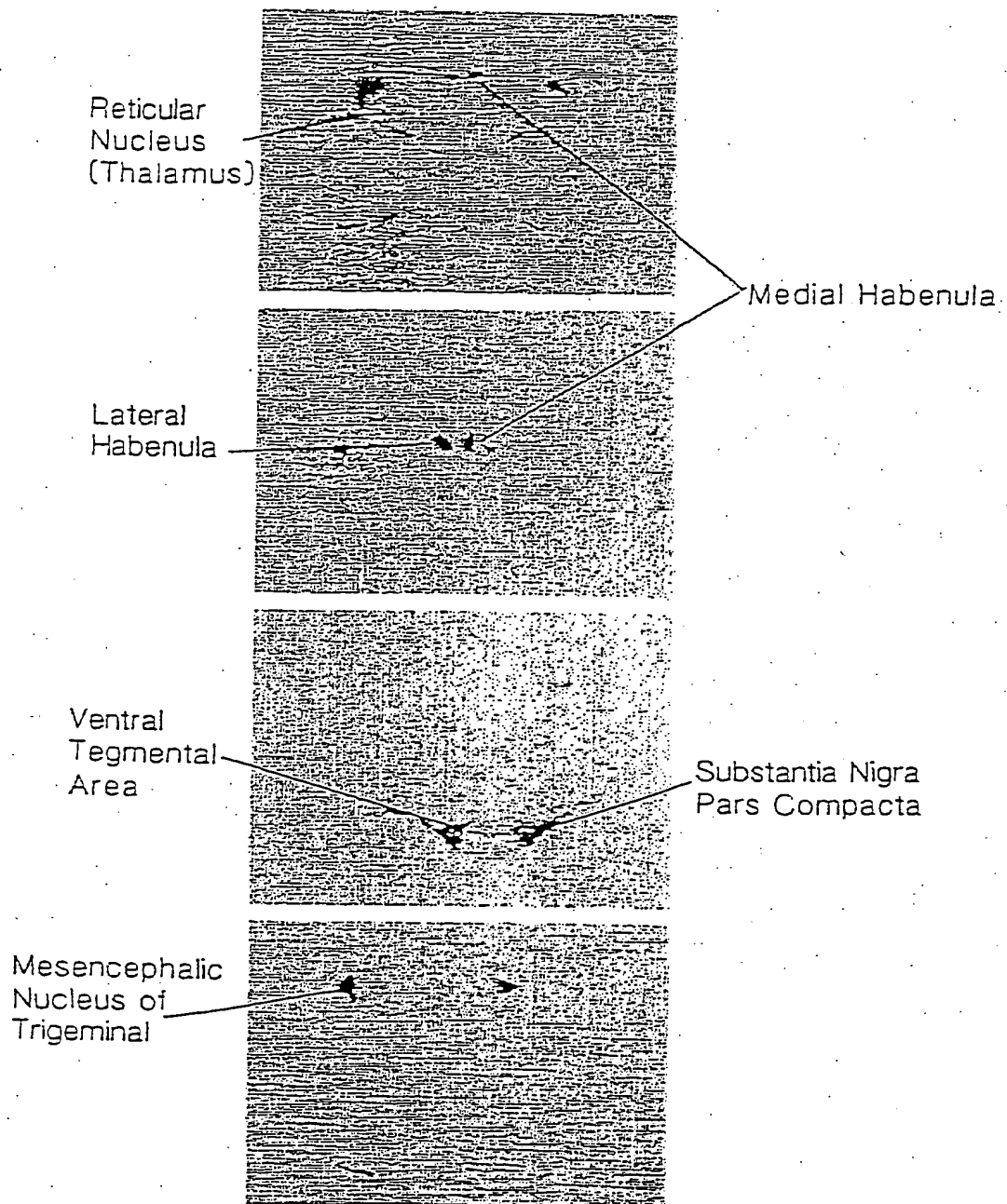


FIG. 24



FIG. 25

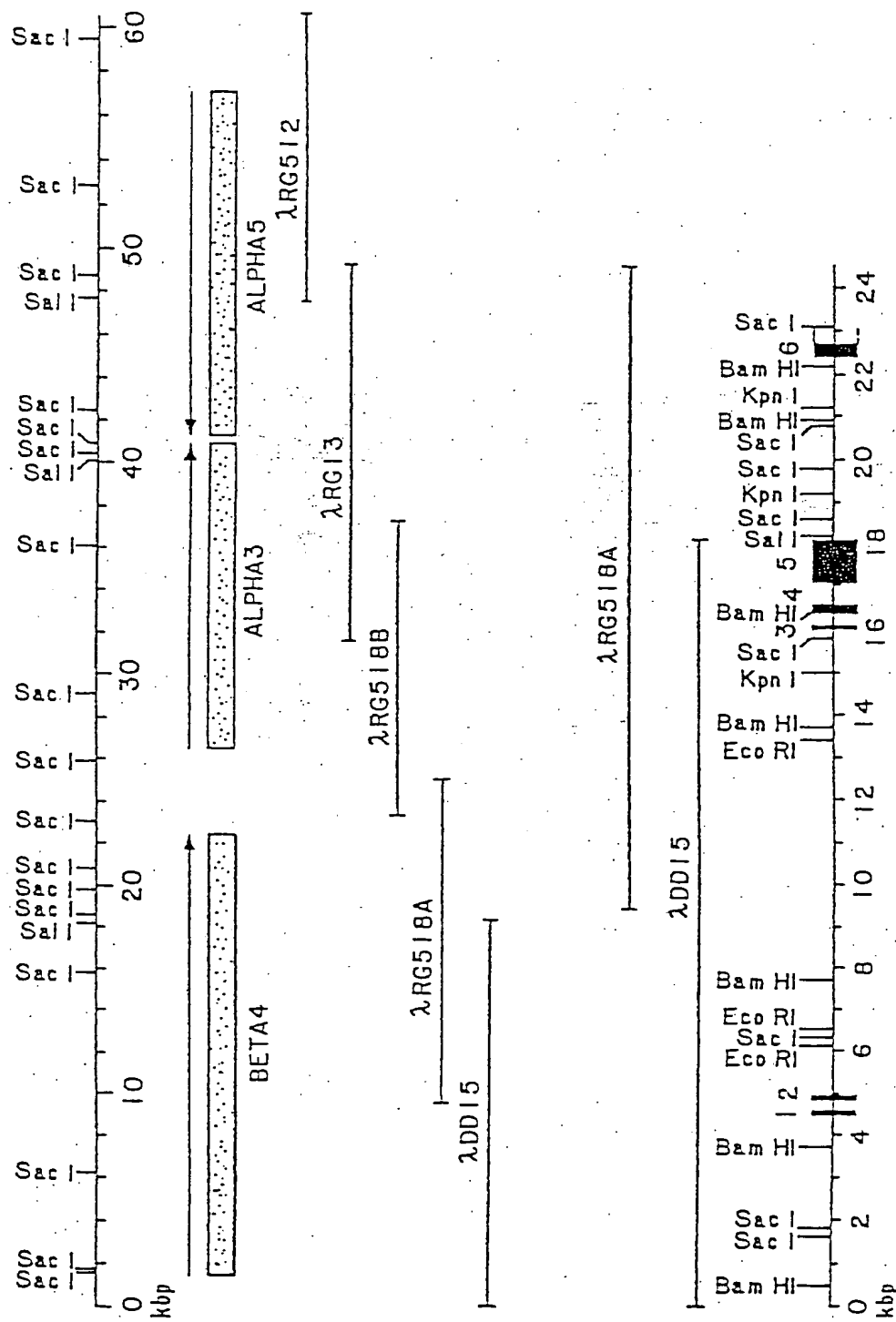


FIG. 26

5'...GCCAACCGGGACAT -120

ACGCTCACTGGCCTTCCATTGTAGAGTGACGGCTGCCACCGGCTGGCC -100
 Met Arg Gly Thr Pro Leu Leu Val Ser Leu Phe Ser Leu Leu Gln Asp -10
 -20
 -40
 -60
 -80
 -100
 -120

Gly Asp Cys Arg Leu Ala Asn Ala Glu Glu Lys Leu Met Asp Asp Leu Leu Asn Lys Thr Arg Tyr Asn Asn Leu Ile Arg Pro
GGG GAC TGC CGC CTG GCC AAC GCA GAG GAG AAG CTG ATG GAT GAC CTC CTG AAC AAA ACC CGG TAC AAC ATC CGC CCA
 1 20 40 60

Ala Thr Ser Ser Ser Gln Leu Ile Ser Ile Arg Leu Glu Leu Ser Gln Leu Ile Ser Val
 GCC ACC AGC TCC TCT CAG CTC ATC TCC ATC CGC CTG GAG CTA TCA TCA TCC CAG CTC ATC AGT GTG
 80 100 120 140

Arg Glu Gln Ile Met Thr Thr Ser Ile Trp Leu Lys Gln
 CGA GAA CAG ATC ATG ACC ACC AGC ATC TGG CTG AAA CAG gtaagtgaact... ..cttaggaaty GAA TGG ACT GAC TAC CGC CTG GCC TGG AAC
 160 180 200 220

Ser Ser Cys Tyr Glu Gly Val Asn Ile Leu Arg Ile Pro Ala Lys Arg Val Trp Leu Pro Asp Ile Val Leu Tyr Asn As
 AGC TCC TGC TAT GAA GGG GTG AAC ATT CTG AGG ATC CCC GCA AAG CGT GTC TGG TTG CCT GAC ATC GTG TTG TAC AAC AA gtgagtgaaca..
 240 260 280 300

...cctaccccag T GCC GAT GCC ACC ACC TAT GAG GTG TCT GTC TAC ACC AAC CTG ATT GTG CGT TCC AAC GGC AGC ATC CAG TGG CTG CCC CCT
 320 340 360 380

FIG. 27A

130 Ala Ile Tyr Lys Ser Ala Cys Lys Ile Glu Val Lys His Phe Pro Phe Asp Gln Gln Asn Cys Thr Leu Lys Phe Arg Ser Trp Thr Tyr
 GCT ATC TAC AAG AGT GCC TGC AAG ATT GAG GTG AAG CAC TTT CCC TTC GAC CAG CAG CAG AAC TGC ACC CTC AAA TTC CGC TCC TGG ACC TAT
 380 400 420 440

160 Asp His Thr Glu Ile Asp Met Val Leu Lys Ser Ala Thr Ala Ile Met Asp Phe Thr Pro Ser Gly Glu Trp Asp Ile Val Ala Leu
 GAC CAC ACG GAG ATT GAC ATG GTT CTT AAG TCG CCC ACG GCC ATC ATG CAT GAC TTC ACC CCC AGT GGT GAA TGG GAC ATT GTG GCC CTC
 480 500 520 540

190 Pro Gly Arg Arg Thr Val Asn Pro Gln Asp Pro Ser Tyr Val Asp Val Thr Tyr Asp Phe Ile Ile Lys Arg Lys Pro Leu Phe Tyr Thr
 CCA GGA CGG ACG ACG GTG AAC CCT CAG GAC CAC CCC ACG TAC TCG GCT ATC ATC CTG GTC ACC TAT GAC TTC ATC ATC AAC CGC AAC GCG CTC TTC TAC ACC
 560 580 600 620

220 Ile Asn Leu Ile Ile Pro Cys Val Leu Ile Thr Ser Leu Ala Ile Leu Val Phe Tyr Leu Pro Ser Asp Cys Gly Glu Lys Met Thr Leu
 ATC AAT CTT ATC ATT CCT TGT GTG CTC ATC ACC TCG CTG GCT ATC ATC CTG GTC TTC TAC CTG CCC TCC GAC TGT GGG GAG AAG ATG ACG CTC
 640 660 680 700 720

250 Cys Ile Ser Val Leu Leu Ala Leu Thr Phe Phe Leu Leu Leu Ile Ser Lys Ile Val Pro Pro Thr Ser Leu Asp Ile Pro Leu Ile Gly
 TGC ATC TCT GTG CTG CTG GCA CTC ACG TTC TTC CTG CTG CTC ATC TCC AAG ATC GTG CCT CCC ACC TCC CTT GAC ATA CCG CTC ATT GGC
 740 760 780 800

280 Lys Tyr Leu Leu Phe Thr Met Val Leu Val Thr Phe Ser Ile Val Thr Thr Val Cys Val Leu Asn Val His His Arg Ser Pro Ser Thr
 AAG TAC CTC TTG TTC ACC ATG GTG GTG CTC GTC ACC TTT TCC ATG GTC ACC ACT GTG TGT GTG CTC AAT GTG CAC CAC CAC TCA CCC AGC ACT
 840 860 880 900

310 His Thr Met Ala Ser Trp Val Lys Glu Cys Phe Leu His Lys Lys Leu Pro Thr Phe Leu Phe Met Lys Arg Pro Gly Leu Glu Val Ser Leu
 CAC ACC ATG GCA TCC TGG GTC AAG GAG TGC TTC CTG CAC AAA CTA CTG CCC ACC TTC CTC TTC ATG AAG CGT CCC GGT CTT GAA GTC AGC CTC
 920 940 960 980 1000

FIG. 27B

[illegible]

CGTTGCTCGGCGCTCGGGGTTATCCGCGAGGCGGCTCGGCGGCGGCGGCTCGGCGGCGGCGGCTCGGCTGCTCTTG
-180 -160 -140 -120 -100

Met Val Gln Leu Leu Ala Gly Arg Trp Arg Pro Thr Gly Ala Arg Arg Gly Thr Ala Gly Gly Leu Pro Glu Leu Ser Ser Ala Ala Lys
ATG GTG CAG CAG CTG GCA GGG CGC TGG CGG CCG ACC GGG GCG CGG ACC GCG GGA GGG TTG CCT GAA CTA TCC TCT GCT GCC AAA
-80 -60 -40 -20 1

His Glu Asp Ser Leu Phe Arg Asp Leu Phe Glu Asp Tyr Glu Arg Trp Val Arg Pro Val Glu His Leu Ser Asp Lys Ile Lys
CAT GAA GAC AGC TTG TTT AGC GAT TTA TTT GAA GAC TAC GAA AGG TGG GTT CGC CCT GTG GAA CAC CTG AGC GAC AAG ATA AAA ATC AAG
10 20 30 40 50 60 70 80 90

Phe Gly Leu Ala Ile Ser Gln Leu Val Asp Val Asp Glu Lys Asn Gln Leu Met Thr Thr Asn Val Trp Leu Lys Gln Glu Trp Ile Asp
TTT GGC CTT GCG ATA TCT CAG TTA GTG GAT GTG GAT GAG AAA AAC CAG CTG ATG Met Thr Thr Asn Val Trp Leu Lys Gln Glu Trp Ile Asp
100 110 120 130 140 150 160 170 180

Val Lys Leu Arg Trp Asn Pro Asp Tyr Gly Gly Ile Lys Ile Ile Arg Val Pro Ser Asp Ser Leu Trp Ile Pro Asp Ile Val Leu
GTG AAA TTG AGA TGG AAT CCT GAC GAT TAT TAT GGT GGG ATA AAG ATT ATA CGT GTT CCT TCG GAC TCC CTG TGG ATC CCA GAC ATC GAT TTG
190 200 210 220 230 240 250 260 270 280

Phe Asp Asn Ala Asp Gly Arg Phe Glu Gly Ala Ser Thr Lys Thr Val Val Arg Tyr Asn Gly Thr Val Thr Trp Thr Gln Thr Ala Asn
TTT GAT AAT GCA GAT GGA CGT TTT GAA GGG GCC AGC AGC AAA ACA GTT GTG AAG TAC TAC AAC GGC ACT GTC ACG TGG ACG CAA
290 300 310 320 330 340 350 360 370 380

Tyr Lys Ser Ser Cys Thr Ile Asp Val Thr Phe Phe Pro Phe Asp Leu Gln Asn Cys Ser Met Lys Phe Gly Ser Trp Thr Tyr Asp Gly
TAC AAA AGT TCT TGC ACC ATC GAC GTT ACC TTT TTC CCG TTT GAT CTC CAA AAT TGT TCC ATG AAA TTC GGC TCG TGG ACA TAC GAT GGA
390 400 410 420 430 440 450 460 470 480

Ser Gln Val Asp Ile Ile Leu Glu Asp Gln Asp Val Asp Arg Thr Asp Phe Phe Phe Asp Asn Gly Glu Trp Glu Ile Met Ser Ala Met Gly
TCC CAG GTT GAT ATA ATC CTA GAG GAC CAA GAT GTC GAC AGA ACA GAC TTT TTC GAC AAT GGA GAG TGG GAA ATC ATG AGC GCG ATG GGG
490 500 510 520 530 540 550 560 570 580

Ser Lys Gly Asn Arg Thr Asp Ser Cys Cys Trp Tyr Ile Thr Tyr Ser Phe Val Ile Lys Arg Leu Pro Leu Phe Tyr Thr Leu
AGC AAG GGG ANC CCG ACG GAC AGC Ser Cys Cys Trp Tyr Ile Thr Tyr Ser Phe Val Ile Lys Arg Leu Pro Leu Phe Tyr Thr Leu
590 600 610 620 630 640 650 660 670 680

FIG. 28A

220 Phe Leu Ile Ile Pro Cys Ile Gly Thr Ser Phe Phe Leu Thr Val Val Val Phe Tyr Leu Pro Ser Asn Glu Gly Glu Lys Ile Ser Leu Cys
 TTT CTT ATT ATC CCC TGC ATT GGG CTC TCA TTT TTT CTG ACT CTG GTT GTT GTC TTC TAT TAT CTC CCT TCA AAC GAG GGT GAA AAG ATT 720
 640
 250 Thr Ser Val Leu Val Ser Leu Thr Val Phe Leu Leu Val Ile Glu Glu Ile Ile Pro Ser Ser Lys Val Ile Pro Leu Ile Gly Glu
 ACC TCA GTG CTC GTG TCT CTG ACT GTC TTC CTT TTT TTT TTT GTA ATC GAA GAG ATC ATA CCA TCA TCT TCC AAA GTC ATA CCC CTG ATT GGG GAG
 740
 280 Tyr Leu Val Phe Thr Met Ile Phe Thr Leu Ser Ile Met Val Thr Val Phe Ala Ile Asn Ile His Arg Ser Ser TCC TCC Thr His
 TAC TTG GTG GTG TTC ACC ACC CTA TCC ATT ATG GTG ACT GTC TTT GGC ATC AAC ATC CAC CAC CGC TCT TCC TCC ACA CAC
 820
 310 Asn Ala Met Ala Pro Trp Val Arg Lys Ile Phe Leu His Lys Leu Pro Lys Leu Pro Lys Ala Asp Arg Tyr Phe Thr
 AAC GCT ATG GCG CCC TGG GTT CGT AAG ATA TTT CTC CAC AAG CTT CCC AAG CTG CTC TGC ATG AGA AGT CAT GCG GAT AGG TAC TTC ACT
 920
 340 Gln Arg Glu Glu Ala Glu Ser Gly Arg Gly Pro Lys Ser Arg Asn Thr Leu Glu Ala Ala Leu Asp Cys Ile Arg Tyr Ile Thr Arg His
 CAG AGA GAA GAA GCC GAG AGT GGG GCT GGA CCT AAA TCT CCG AAC ACT TTG GAA GCC GCA GCG GAT TGC ATT CGC TAC ATC ACG AGG CAC
 1000
 370 Val Val Lys Glu Asn Asp Val Arg Glu Val Val Val Glu Asp Trp Lys Phe Ile Ala Gln Val Leu Asp Arg Met Phe Leu Trp Thr Phe Leu
 GTC GTG AAA GAG AAC GAC GTC CGC GAG GAT GAT GAA GAT TGG AAA TTC ATA GCC CAA GTC CTT GAT CGG ATG TTT TTG TGG ACG TTT CTT
 1100
 400 Leu Val Ser Ile Ile Gly Thr Leu Gly Leu Phe Val Pro Val Ile Tyr Lys Trp Ala Asn Ile Ile Val Pro Val His Ile Gly Asn Thr
 CTG GTG TCA ATC ATT GGG ACT TTA GGG CTT TTT GTT CCT CCT GTT ATT TAT TAT AAA TGG GCC AAT ATA ATA GTC CCA GTT CAC ATT GGA AAC ACA
 1200
 420

FIG. 28B

Ile Lys
 ATT AAG TGA AACCAAGAAATTACCCTGTGGATTAGTGAGCAGTCAATGCAGGCCTTAGGACATGTATGCTGTTATGGAAATGTGAAGGTAGTTACAATTTGACATAGGCTATAACA
 1280 1300 1320 1340 1360 1380
 GATTAGCAATTTCTAACATTGGCTTAATGTTGTCCATTAGAACTGCAGTAATAACCTCAATAGCAACAACACATTGGTCTGCCTGCACCTAGTGAAGGCCTAGCATCTGCATCCTGGCAA
 1400 1420 1440 1460 1480 1500
 ACCCTACCAATTTGCAACCAATGATGAAGGCCATCCTTGGAGTGTCTGGGAAACTCAACTGTATTGGAAGACTATTAAAACTCCCCCAATTTAGTAGGAACATATATATGTGTGGTT
 1520 1540 1560 1580 1600 1620
 TTGAATTTTCAGAAATGGGTCTTTGGGCTTTGTTAAATTTGCTGGGCTAGCACAAACCTCCTGAGTAGCTGGGACCATGGGTGTGCTGCACCTTTGCCCTGTTCTGTATTCACAGATATA
 1640 1660 1680 1700 1720
 AAATACATCATTATTTATAGGAGGTAGGCCCAATTACTTGGGTTTAAATAAACCTTAATGTCAGTTAGGTTTAAATTAATTAACCTTATGTACAGTAAATGTTCTATTGCTGTGAMGAGACATC
 1760 1780 1800 1820 1840
 ATGACCATCACTCTTATAAAGAAACATTTTCATCAGTGTCTGGCTTACAGTTTTTGGAGTTTAGCCAAATTATCACAGTGAAGCATGATAGCATCCAGGTAGACATAAATGCTGGATCC
 1880 1900 1920 1940 1960
 AGGAGTTCTCTACATCTGGATCAGCAGGCGAGGAGAGAGAGGCCACTGGACCTGGCTTGAGCATCTGAAACCTCAAAAGCCCCACCTCCAGTGACACACACTTCCCCCAACAAAGGCC
 2000 2020 2040 2060 2080
 ACACCTCCTAATAGTGCCACTTGGCTGGTGATCAAGCATTCAGTCTATGGGTCTACGAGAGGCCAATTCCTTAITCAAACCCACACTTAATAGGATGCTATTCTTTACTGACATTTTAAATAAG
 2100 2120 2140 2160 2180 2200
 CGACAAATGGTAACTAGAAACATCGTAGGCCCACTTTACTCTTTTATATGTTAATGGAATGGCTTTTATATTAAGTATTTTACAGCCTATCTGMAAACATGTAACAGGCAACTCCCTG
 2220 2240 2260 2280 2300 2320
 CAGACACATTTCTTGTAATGACTTTTATATCTCTGCAGTGGCATGTTCTGGTAATGACTTCAGTCTCTGCTGCCACACTCTCCGGTTAGTAGAGCCATTTATGTACATCGCGTATCCCTG
 2340 2360 2380 2400 2420 2440
 ATTTACAGAGCAACTGTGCAGTTGCACAGGTTCCCACTCAAAATGGGATGCCATGACTCTGTCTGGATAAATTTCTGTGGAAACCACTTCTGAGCTGGATACGGTGCCTCATACCTGTCA
 2460 2480 2500 2520 2540 2560
 TGTCTACTTTACAGGAGGCGAGGCGAGGGGAAATTGCTGTGAGTTGTTGGTCAGCCTGGGTTACATATGAGACCCCTGTCTCAGAAACCAACAAACAACTTCCCCCTGTGAGTTGATATAG
 2580 2600 2620 2640 2660 2680
 CACACTGTCAATCCGAATTGGGGATCCTCTAGAGTCAAGCTTGGGCTAAATCATATGAGTGGTTCATAGCTGTTTCCCTGTGTGAATTTGTTATCCG
 2700 2720 2740 2760 2780

FIG. 28C

B1TA2 HLACHAGHSHALFSLSLWLCGVLGDTTEERLVEHLLDPYRHNKLRIPATHGSELVTVQLHVSQAQLISVHEREQIHITHVMTQEMEDYRLTWKPEDFDHMK
 B1TA3 HTGFLRVFLVLSATLSGSWVTLTATAGLSSVAEHEDALLRHLFOGYQXWVPVLSNDIIXVYFGLXISQLVDVEKNQMLHTNVMWKQEWTDQKLRHNPPEEYGGIN
 B1TA4 HRTPLDLLVSLFSLQOGDCRLANAEEKLMDDLLNKTRYNNLRPATSSQLISIRLELSQLISVHEREQIHITHVMTQEMEDYRLTWKPEDFDHMK
 ——— SIGNAL FIFTIDE ———

B1TA2 KVLPSKHIWLPDVVLYHNADGHYEVSYSHAVVSYDGSIFWLPAPAIYKSACKIEVKHFFPDQONCTHKSRSWYDORTEIDLVKSDVASLDOFTPSGEWDIIALPG
 B1TA3 SIKVPSESLWLPDIVLFEHADGRFEGSLMKAIVKSSGTVSWTPPASYSKSSCTHDTVTFPPDRONCSHKFGSWTVOGTHVDLILINEHVDRKDFPFONGEWEILNAKG
 B1TA4 ILRIPAKRVMLPDIIVLYHNADGHYEVSVYTHIVRSNGSIQWLPAPAIYKSACKIEVKHFFPDQONCTHKSRSWYDORTEIDLVKSDVASLDOFTPSGEWDIIALPG

B1TA2 RRHNPDDOS TYVDITYDFIIRRRKPLFYTHLIIPCVLITSLAILVFLYLPSCGEXMTLCISVLLALTIVFLLLSKIVPPTSLDVPVLVGVKYLHFTHVLVTFISVTSV
 B1TA3 HKGHRREGFYSPFVTVSVLRRRLPLFYTLIIIPCLGSLFTLVLFYLPSCGEXMTLCISVLLALTIVFLLLSKIVPPTSLDVPVLVGVKYLHFTHVLVTFISVTSV
 B1TA4 RRTVNPQDP SYVDVTVDFIIRRRKPLFYTHLIIIPCVLITSLAILVFLYLPSCGEXMTLCISVLLALTIVFLLLSKIVPPTSLDVPVLVGVKYLHFTHVLVTFISVTSV
 ——— MSR I ——— ——— MSR II ——— ——— MSR III ———

B1TA2 CVLHVHRSPTT HTHAPWVKVVFLEKPLTLFLQOPNRHRCARQRLRRQREGEAVFTRGPAAADPCSVGPCSCG
 B1TA3 FVIVHHRSSSTYBPHAPWVKRFLQRLPWLCHKDPDRFSFPDGKESDTAVRGKVSXKQOTPASDGERVLVAFLEK
 B1TA4 CVLHVHRSPT HTHASHVKECFLHKLPTTLFMKRPGLVSLVRVPHPSQLHATADTATASALGTPSPNLYGSSHYFVNPVPAAPKSAVSSHTAGLPRDARLRS
 ———

B1TA2 LREAVDGVRFIADHHRSEDDOSVREDWVKYVAHVIDRLFLMIFVFCVGTVGMLFLOPLFQNYTATITLHPDHSAPSSK*
 B1TA3 ASESIRYISRHVKKHEHFIQVQVQDNKFAQVLDRIFLHLFLIASVLGSLIIFIPALKMHIHRFH*
 B1TA4 SGRFREDLQEALEGVSFIAOHLESDDRDOSVIEDHRTVAHVVDRLFLMIFVFCILGTHGLFLPLPLFOIHAPS KDS*
 ——— MSR IV ———

FIG. 29

ALPHA2	HTLSHALQFWTHLYLWCLLLVPAVLTQOQSHTHAEDRLFKHLFGYNRHARVPHTSDVVRVGLSIAQLIDVDEKQNMHTTHVHLKQEWNDYKLRWDPAE
ALPHA3	HGVLLPPLPLSHLMLVLMHLLPAASASEAEHRLFYLYFEDYNEIIRVANVSHRVIIOFVSHSQLVKVDEVHQHETHLHLKQIWHQYKLRKPPSD
ALPHA4	HEIGGPGAPFPLLLPLLLGLTGLPASHSHIETRAHAEERLKLRLFGYHKWSRPVCHISDVVLVRGLSIAQLIDVDEKQNMHTTHVVKQEWNDYKLRWDPGD
ALPHA5	HVQLLAGHWRPTGARRGTAGGLPELSAAKHEDSLFRDLFEDYERHWRPVEHLSDKIKIKIGLAISQLVDVDEKXQLGHTTHVHLKQEWIDVKLWHNPD
	----- SIGNAL PEPTIDE -----
ALPHA2	FQNVTSURVSEHETHIPDIVLYNHADGEFAVTHNTKALHFTGTVHVHPPAIYKSSCSIDVTFFPDQONCKMKFGSWTYDKAKIDLEQHENTVDLKDYMESGEWA
ALPHA3	YOGVEFHRVPAEKIMKPOIVLYNHADGDFQVDDTKALLKYTGVEVTHPTPAIFKSSCKIDVTFFPDQONCTHKFGSWTYDKAKIDLVIGSSHMLKDYMESGEWA
ALPHA4	YENVTSIRIPSELIWRPOIVLYNHADGDFAVTHLTKAHLFYDGRVQHTPPAIYKSSCSIDVTFFPDQONCTHKFGSWTYDKAKIDLVSIHNRVDDQLDFMESGEWV
ALPHA5	YGGIKIIRVPSDSLHNPDIIVLFDNADGRFEGAS TKTVVRVNGTVTHTQTPANYKSSCTIDVTFFPDQONCHMKFGSWTYDGSQVDIILEDQDVDRDTDFDNGEWE
	----- ** -----
ALPHA2	IINATGYNSKKYDCCAEIYVDVTVYVIRRLPLFYTIINLIIPCLLISCLTVLVYLPSECGEKITLCISVLLSLTVFLLLIITEIPSTSLVPLIGEYLLFTMHF
ALPHA3	IIKAPGYKHEIKYNCCEEIIYQDITYSLYIRRLPLFYTIINLIIPCLLISCLTVLVYLPSECGEKITLCISVLLSLTVFLLLIITEIPSTSLVPLIGEYLLFTMHF
ALPHA4	IYDAGVGYNTRKYECCEAEIYVDITYATIIIRRLPLFYTIINLIIPCLLISCLTVLVYLPSECGEKITLCISVLLSLTVFLLLIITEIPSTSLVPLIGEYLLFTMHF
ALPHA5	IHSANGSKGHRDTSCCH YPIITYSVIKRLPLFYTLFLIIPCIGLSCLTVLVYLPSECGEKISLCTSVLVSLTVFLLVIEEIPSSSKVPLIGEYLVFTMHF
	----- MSR I ----- MSR II ----- MSR III -----
ALPHA2	VTLSIVITVFLVHVHRSPTTHNPN WVRVALLGRVPRHLHNRPLPPHELHGSDDLKLSPSYHMLETHMDAGEREREETEEEEEDENICVCAGLPDSSHGVLYG
ALPHA3	VTLSIVITVFLVHVHYRTPTTHPT WVKAVFLMLLPVHPTHTPTSGEDTPKTRTFYGAELSLNLCFSRADSKSKCEGYPCQDGTGCGYCHRRVVKISHFSANL
ALPHA4	VTLSIVITVFLVHVHRSPTTHTPA WVRVFLDIVPRLLEFKRPSVVDKNCRRLLIESHHKANAPRTHPEFVGEPCILSDICNOGLSPAPITFCNPTDTAVETQP
ALPHA5	VTLSIHVTVFAINIHRRSSSTHNAHAPVVRKIFLHKLPLKLLCHRSADRYFTQREAEESGAGPKSRNT

ALPHA2	HGGLHLRAHEPETKTPSQA
ALPHA3	TRSSSESSEVNAV
ALPHA4	TCRSPPLEVPDLXTSEVERKASPCSPGSCPPPKSSSGAPHLIKARSLSVQHVPSQEAADGIRCHRSRSDYCVSQDGAASLADSKFTSSPTSLKARPSQLPVSDQ
ALPHA5	
ALPHA2	SZILLSPOIKALEGVHYIADRLRSEDADSSVKEDMKYVAHVVDRIFLMHFIIVCFGLGTIGLFLPPFLAGHI*
ALPHA3	SLSALSPEIKKATQSVKYIAZNNHKAQHVAKIQQDDMKYVAHVVDRIFLVWVFLVLCILGTAGLFLQFLHARDOT*
ALPHA4	ASPCKCTCKEPSVSPVTVLKAGGTAKAPPQHLPLSPALTRAVEGVQYIADHLKAEEDTFSVKEDMKYVAHVVDRIFLWHFIIVCLEGTVGLFLPFLAGHI*
ALPHA5	LEAALDCIRYITRHHVVKENDVREVVEDMKFIQVLDHRLHFLMTFLVLSIIGTLGLFVVPVFLNGPI*
	----- MSR IV -----

FIG. 30

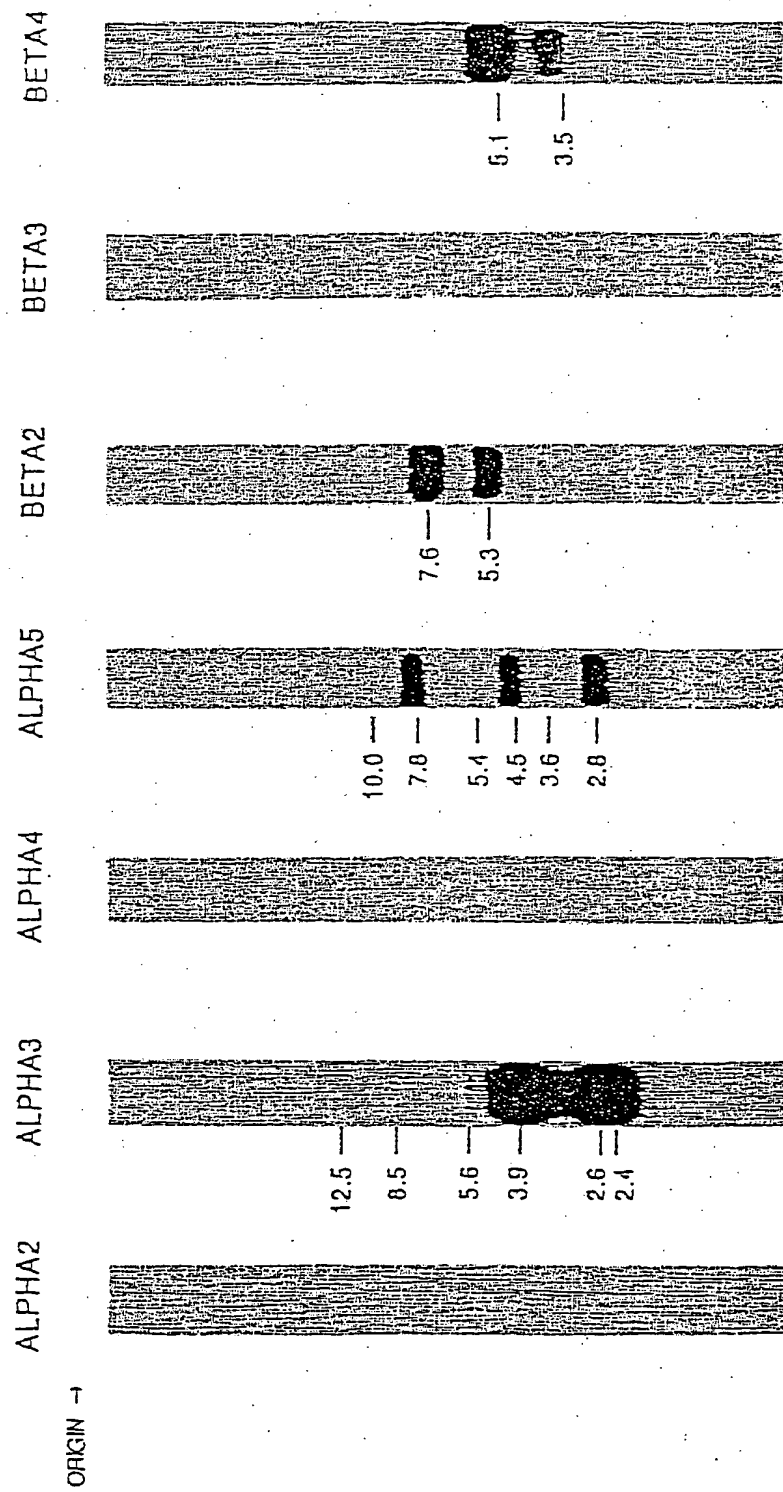


FIG. 31

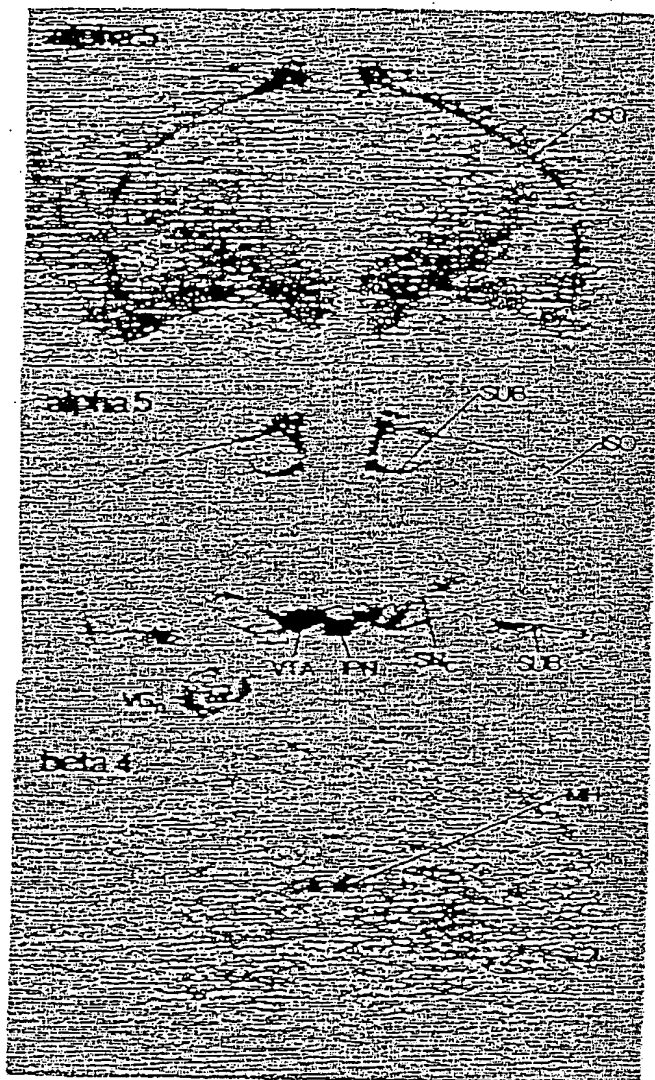


FIG. 32



North2South Direct Stringency Wash

Notes/Conditions	Tips	Formula	References	Links/Products	Contents
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North2South Direct Stringency Wash

Stringency wash and final wash buffers are prepared from 20X SSC and 10% SDS provided in the North2South Direct Kit. Buffers should be prepared fresh using clean labware and quality-grade water (nuclease-free). Using old wash buffers risks bacterial contamination common in dilute buffer systems without preservative especially when incubated at high temperature. Prepare enough stringency wash and final wash buffer solution for 3 washes of 20 ml/100 cm² each. Wash volumes of 40 ml are sufficient when using the 50 ml centrifuge tubes. Stringency may be controlled by buffer salts or temperature. The standard North2South Direct Protocol recommends using a low stringency wash buffer, 2X SSC, 0.1% SDS warmed to the same temperature used for hybridization. High stringency wash buffer, 0.5X SSC, 0.1% SDS, or ultrahigh stringency wash, 0.1X SDS, 0.1% SDS may be used if needed. It is important to verify buffers and incubator temperature with calibrated thermometers. Use of lower temperatures may cause background and nonspecific detection; higher temperatures may wash off target. Low stringency may be more important for smaller probes. High stringency conditions may be required for plaque lifts or crude samples. Be aware the dry and water bath incubators differ in time required to bring buffer to temperature. The stringency wash buffer may require 1-2 hours to come to temperature for larger volumes. If rapid warming is required, the buffer can be warmed in 40 ml aliquots in 50 ml centrifuge tubes. After hybridization, transfer the blot to a clean wash container filled with stringency wash buffer. Wash 3 x 5 minutes with the stringency wash buffer at the same temperature used for hybridization. Do not exceed 15 minutes or reduced signal may occur. Next, wash 3 x 5 minutes with 2X SSC at room temperature. Nonspecific signal is rarely observed. However, stringency wash buffer may be altered if desired.

Notes/Conditions

Nucleic acid thermal hybrid stability is expressed in terms of its melting temperature T_m . Factors that influence the hybridization and washing include temperature, ionic strength, destabilizing agents, mismatched base pairs, duplex length and viscosity and base composition. Stringency washes are controlled by altering temperature, salt concentration and destabilizing agent concentration. High salt concentration favors hybridization reactions. Optimal hybridization is reported at 1.5 M Na⁺ (Labfax). Lowering the salt or increasing detergent concentration may reduce nonspecific hybridization and background. Increasing temperature should also decrease nonspecific hybridization and background. ([back to top](#))

Tips

- Use nuclease-free grade water (filtered or DEPC) for wash buffer preparation.
- Normal wash conditions use 2X SSC, 0.1% SDS (at hybridization temperature) followed by 2X SSC (room temperature). High stringency washes (i.e., 0.1-0.5X SSC, 0.1% SDS) may reduce background for crude samples or plaque lifts. Low stringency (i.e., 5X SSC, 0.1% SDS) may help smaller probes or low T_m probes to remain hybridized.
- Temperature as well as salt concentrations may be adjusted to control stringency washes. Lowering the temperature may favor nonspecific hybridization while raising the temperature may wash hybridized probe from target.
- Do not exceed 3 x 5 minute washes. The longer exposure to heat in the absence of stabilizers can damage the enzyme.
- Make sure the hybridization oven and buffers are at appropriate temperature by measurement with a calibrated thermometer. ([back to top](#))

Formula

20X SSC 0.3 M trisodium citrate, 3 M NaCl, pH 7, 0.2 μ m filtered
20X SSPE provides better buffering for formamide based systems. This system is not useful for North2South Direct due to the fact that HRP is not stable in organics. ([back to top](#))

References

1. Brown, T.A. (1991). Molecular Biology LabFax. Bios Scientific Publishers Limited.
2. Sambrook, J., *et al.* (1989). Molecular Cloning: A Laboratory Manual. 2nd ed, Cold Spring Harbor Laboratory Press.
3. Ausubel, F.M., *et al.* (1995). Current Protocols in Molecular Biology. John Wiley & Sons, Inc. ([back to top](#)).

Links/Products

20X SSC and 10% SDS? Will we carry separately? Other? ([back to top](#))
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Other:

[Endogen](#) [Pierce Nucleic Acid Technology](#) [HyClone](#) [Atos Medical](#)

DIG Reagents and Kits for Non-Radioactive Nucleic Acid Labeling and Detection



last update September 7, 2000

Hybridization stringency

A very important factor for a successful blotting experiment is the stringency of the hybridization conditions. If the stringency is too low, unspecific hybridization may occur. On the other hand, if the stringency is too high only weak or even no signals may be observed.

For any hybridization, stringency can be varied by manipulation of three factors: temperature, salt concentration, and formamide concentration.

Factor	Influence
Temperature	High temperature increases stringency Low temperature decreases stringency
Salt concentration	High salt decreases stringency Low salt increases stringency
Formamide	Decreases melting point of DNA, thus lowering the temperature at which a probe-target hybrid forms. Thus, rising amounts of formamide increase stringency.

Which hybridization conditions are optimal strongly depends on the type and the GC content of the hybrids. RNA:RNA and RNA:DNA hybrids will require higher hybridization temperatures than DNA:DNA hybrids. In general, the relative strength of different hybrids is **RNA:RNA hybrids > RNA:DNA hybrids > DNA:DNA hybrids**. As a rule of thumb, for mammalian targets containing 40% GC, the optimal hybridization temperatures in the presence of DIG Easy Hyb or 50% formamide are:

For This Type of Hybrid	T _{opt} is
DNA:DNA	37 - 42°C
DNA:RNA	50°C
RNA:RNA	68°C

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